Backbone Amide Linker in Solid-Phase Synthesis

Ulrik Boas

National Veterinary Institute, Technical University of Denmark, Bülowsvej 27, DK-1790 Copenhagen, Denmark

Jesper Brask

Novozymes A/S, Krogshoejvej 36, DK-2880 Bagsvaerd, Denmark

Knud J. Jensen*

Faculty of Life Sciences, Department of Natural Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

Received April 24, 2007

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* To whom correspondence should be addressed. E-mail: kjj@life.ku.dk.

1. Introductions

1.1. Brief Introduction to Solid-Phase Synthesis

In 1963, Merrifield reported in a seminal paper the concept and first implementation of solid-phase peptide synthesis (SPPS).[1] Since then, solid-phase synthesis has evolved into a highly efficient set of techniques for the preparation of numerous peptides, small proteins, oligonucleotides, and nonoligomeric organic molecules. It has been crucial in the development of combinatorial and high-throughput chemistry as well as many other research areas. While the term "solid-

10.1021/cr068206r CCC: $71.50
Published on Web 03/16/2009

Ulrik Boas (left) received his M.Sc. degree in organic chemistry from the University of Copenhagen in 1996. After a period as a research scientist in the biotech industry, he received a Ph.D. degree in organic and macromolecular chemistry in 2002 from the University of Copenhagen, under the supervision of Jørn B. Christensen and Knud J. Jensen. He is currently an associate professor at the National Veterinary Institute, Technical University of Denmark. His fields of interest are method development in bioorganic chemistry and biomedical use of dendrimers and branched molecules as multivalent scaffolds in immunology and protein chemistry.

Jesper Brask (right) received his Ph.D. degree from the Technical University of Denmark in 2002 on the subjects of protein *de novo* design and solid-phase peptide synthesis, under the supervision of Knud J. Jensen. Since 2002, he has been employed by Danish biotech company Novozymes, presently holding a position as science manager. Current research interests are focused on biocatalysis, enzymology, and immobilization chemistry.

Knud J. Jensen (center) received dual degrees in philosophy and chemistry from the University of Copenhagen. He obtained a Ph.D. degree from the University of Copenhagen in 1992 for research at the Carlsberg Laboratory under the supervision of Morten Meldal. He then spend four years as a postdoctoral fellow at the University of Minnesota in research group of George Barany. He returned to Denmark in 1997 as an assistant professor at the Technical University of Denmark, where he established an independent research group. He became a research associate professor at the same institution in 2000. In 2001 he was appointed associate professor at the Royal Veterinary and Agricultural University, which in 2007 merged with University of Copenhagen. Also in 2007, he was promoted to full professor at the University of Copenhagen. His research covers a broad range of topics in synthetic bioorganic chemistry and nanobioscience centered around solid-phase chemistry, focusing on synthetic peptides and carbohydrates, and designer proteins as well as the study of their nanoscale properties.

phase synthesis" is commonly used, "matrix-assisted synthesis" has been suggested as a more precise terminology, as the polymers commonly used are not solids.[2] The main characteristics of solid-phase synthesis are that (i) the first building block is attached (anchored) to a matrix, which can be filtered, (ii) repeated cycles of chemical transformations (especially deprotection and coupling) are performed, also by automation, and (iii) most often the final product is released from the matrix, but by proper choice of chemistry the final product can also be deprotected while it remains attached to the support. In Merrifield's original design for solid-phase peptide synthesis, the growing peptide chain was anchored through the C-terminal carboxyl while elongated from the N-terminal. However, many biologically active peptides are either C-terminal modified or cyclic, and their synthesis would be difficult or impossible through C-terminal anchoring. Since then, innovative methods for side-chain anchoring have been reported. In the mid-1990s, a new, general methodology for anchoring through backbone amide nitrogens was described, the so-called Backbone Amide Linker (BAL). The present review provides a comprehensive survey of the by now widely used BAL chemical methodology, which extends far beyond applications in peptide chemistry.

1.2. General Introduction to Linkers

In Merrifield's original approach, the C-terminal amino acid of the peptide was attached directly to the support, but since then linkers (handles) between the support and the growing peptide chain were developed as go-betweens, which allowed release of the peptide after completion of the peptide chain assembly. In the words of Songster and Barany: "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".[3]

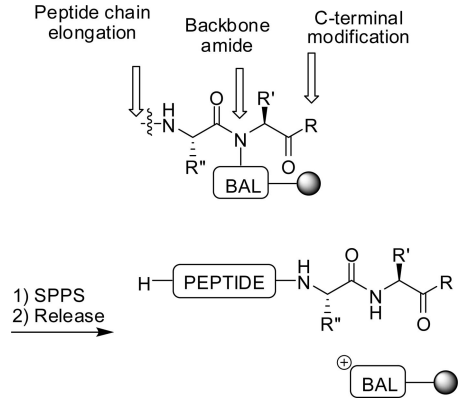
The attachment to the matrix, most often a polymeric support, is a crucial part of solid-phase synthesis.[4] The first building block has to be attached efficiently, the linkage has to be stable to the subsequent chemical transformations, and conditions for release from the support should be compatible with the final products. Most commonly, the assembled products are released under acidic conditions, typically by treatment with "cocktails" containing trifluoroacetic acid (TFA) or HF. However, linkers have also been developed to release the final product by treatment with base or fluoride, nucleophilic displacement, photolysis, oxidation/reduction, alkylation followed by nucleophilic displacement, etc.

Linkers, which upon treatment with concentrated TFA release amides by C−N bond cleavage, have in most cases been designed from substituted benzyl, benzhydryl, and trityl derivatives as "core structures", with addition of substituents to fine-tune the acid-lability.[5] Development of new linkers for solid-phase synthesis continues to be of great interest, and new core structures, in addition to the above-mentioned, could serve as starting points for the design of whole new families of linkers.

1.3. Backbone Amide Linker (BAL) Concept

The Backbone Amide Linker (BAL) strategy was developed independently by several groups in the mid-1990s to address the synthesis of C-terminal modified peptides and to meet other synthetic challenges.[6,7] In this strategy, the first amino acid is anchored by reductive amination (in the following abbreviated RA), followed by acylation of the newly formed secondary amine. Thus, the growing peptide chain is anchored *not* through the C-terminal carboxyl but through a backbone amide nitrogen giving access to, in principle, any C-terminal modification (Scheme 1). The BAL

Scheme 1. Schematic Outline of Solid-Phase Peptide Synthesis by a BAL Strategy[6]



concept for peptide synthesis was presented in lectures at two international conferences in 1995.[6] In 1995, Ellman and co-workers published a seminal report on the solid-phase synthesis of benzodiazepines.[7] In 1997, Fivush and Wilson,[8] Swayze,[9] and Sarantakis and Bicksler[10] reported related linkers. The BAL concept took inspiration from backbone amide *protection* in peptide synthesis, originally described by Weygand et al.[11] and then further developed by Sheppard and others.[12] Furthermore, two conceptually related approaches had been reported, however, without full utilization as a general BAL approach.[13] Early work on transformation of solid-supported aldehydes was reported by Leznoff and Wong.[14]

In the first implementation of the BAL strategy, amino acid derivatives were attached by convenient and reliable RA to support-bound 5-(4-formyl-3,5-dimethoxyphenoxy)-valeric acid, forming a trialkoxybenzylamine linkage. This aldehyde, nicknamed "PALdehyde", had previously been reported as an intermediate in the synthesis of the PAL (Peptide Amide Linker) handle.[15] Also, RA of this compound with trityl amine[16] and simple alkyl amines had been reported.[17] A defining feature of the BAL strategy is that the linker precursor is an aromatic aldehyde. RA of aromatic aldehydes will form a benzyl-type linkage, which, upon eventual acidolytic cleavage, generates a relatively stable carbenium ion (carbocation).

The BAL strategy has since also been applied to the synthesis of oligosaccharides (section 4) and nitrogen-containing nonoligomeric organic molecules (section 5). A variety of BAL handles, which extend beyond the benzyl core, have been prepared and applied in solid-phase synthesis (section 2).

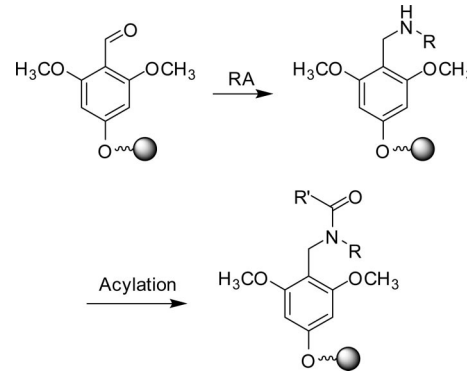
1.4. General Aspects of BAL Chemistry

In the following sections, conditions for individual reactions are mentioned. However, as an introduction, a few general considerations on reagents, solvents, procedures, and mechanisms are required here. At the end of this review, there will be a short summary and overview of protocols.

1.4.1. Anchoring of the First Residue: Reductive Amination

Common for all BAL-type handles is that the first residue is anchored by RA to the aldehyde linker precursor. Hence, the linker precursor is the electrophile, whereas the incoming first "building block" acts as the nucleophile. The only other widely used class of electrophilic linkers are trityl based.

Scheme 2. Two Key Steps in BAL Chemistry, Illustrated in the Trialkoxybenzyl System: Reductive Amination (RA) and Acylation of the Formed Secondary Amine



Merrifield chloromethyl polystyrene resin is also a solid-phase bound electrophile, which has been used extensively for anchoring the first amino acid through the carboxyl;[1] however, in modern applications, it is more a "base resin" for the anchoring of linkers. Less common are benzyl halide linkers, e.g., chloro analogues of Wang and SASRIN linkers[18] as well as the THP linker for release of alcohols.[19] Alternatively, the Mitsunobu reaction has been used to esterify benzyl alcohol type support with Fmoc-amino acids.[20] Benzhydryl chloride type linkers have also been reported for BAL-type chemistry.[21] While many electrophiles are often prone to decomposition, e.g., hydrolysis of benzyl and trityl halides, aldehydes are relatively stable electrophiles, even in the presence of water, as the formation of hydrates is reversible. Aldehyde-containing reagents are, therefore, a very convenient class of eletrophilic linker precursors.

Two different tactics have been used in the implementation of BAL chemistry (see Scheme 2). By far the most predominant is to first attach the aromatic aldehyde to the support and then perform the RA on-resin. The alternative—much less frequently used after its initial report in the original BAL paper[6]—is first RA of the linker precursor with the amino acid derivative in solution, followed by protection of the secondary amine and then coupling of the conjugate to the support, typically by amide formation.

Because the attachment of the amine most commonly occurs by RA, the first residue for peptide synthesis would, thus, typically be an amino acid derivative with a free amino group and a protected or modified carboxylic acid moiety. The amino acid can be used as either the free amine or as the salt, e.g., a HCl salt. In most cases, it is essential to perform the reaction under slightly acidic conditions, typically using 1% (v/v) AcOH; however, often the amino acid as the HCl salt provides sufficient acid catalysis. MeOH is the preferred solvent for RAs, but neat MeOH does not sufficiently swell polystyrene (PS) resins. DMF (and NMP) is an excellent solvent for resins, and RAs have been conducted in this medium, especially in the presence of added AcOH. In solution, RA in DMF easily leads to the undesired *N*,*N*-dialkylated product, while in methanol the monoalkylated product is favored.[6] However, on the solid phase, likely due to the excess of amine used and some degree of site−site isolation[22] (especially for relatively low-loading resins), monoalkylation is favored over unproductive dialkylation.[6] Reducing agents used have been mainly $NaBH_3CN$ and, to some extent, $NaBH(OAc)_3$. Anchoring by RA is normally performed as a one-pot reaction, which combines imine formation with reduction. Under these conditions, anchoring

is generally racemization-free. However, when the reaction is performed as a two-step protocol, where the imine is first formed at 60 °C, followed by reduction, high levels of racemization have been observed.[6,7] Often the amine and reducing agent is used in 2- to 10-fold excess, if feasible, with typical reaction times being several hours at room temperature.

An important factor for the RA step, and presumably also for other subsequent steps, is the distance from the solid phase, which in the case of PS resins may have a strong disruptive effect on the polar solvent cage required for a great variety of synthetic procedures in peptide synthesis. For di- and trialkoxybenzaldehyde linkers, Bui et al. found that not only the degree of substitution on the aromatic ring and the corresponding steric hindrance but also the properties of the solid phase and the presence of a spacer between the linker core and the solid phase were important factors to ensure good RA yields.[23] Their study concluded that the presence of a five-carbon spacer between the linker core and the solid phase greatly improved the yield of released substrate from a Synphase Crown resin (i.e., a polypropylene stick grafted with polystyrene and functionalized with a linker for SPS).[24] Upon derivatization of Merrifield chloro- or aminomethylated PS resins, the effect of the spacer was less pronounced. In the latter case, the degree of substitution on the linker core seemed to be of importance, resulting in higher RA yields on the less-substituted benzaldehyde motif. However, the conditions for the subsequent acylation reaction may also have a significant impact on the total release yield. Bui et al. used DIPCDI/HOBt for the subsequent acylation.[23] This mixture may give poor yields in acylation of the trialkoxybenzylalkylamine, which also may be the reason for the low yield of released substrate.

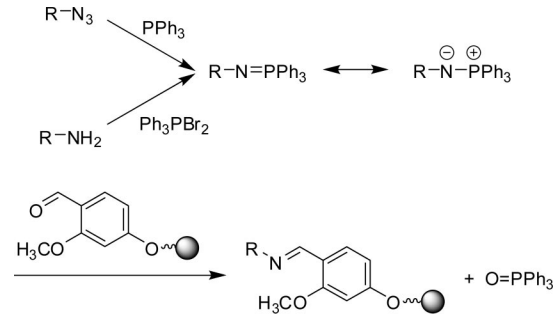
In this context, it is worth mentioning that applying microwave irradiation during RA of the aldehyde moiety of mono- and trialkoxy-based BAL handles recently was found to considerably increase efficiency and reduce reaction times from overnight to a few minutes.[25] Also, the efficiency of the subsequent acylation step and the repetitive coupling and deprotection steps can benefit significantly from microwave heating.

Another less frequently used strategy for increasing the efficiency of the RA step on the various benzyl BAL linkers is the use of additives, e.g., various dehydration agents such as trimethylorthoformate (TMOF), or the use of synthetic amino equivalents with high "oxophilicity". As an example of the latter strategy, the nucleophilicity of the amine substrate toward oxygen-containing electrophiles may be enhanced by converting the amine or the azide to the corresponding iminophosphorane by the reaction with a phosphonium or phosphine compound, respectively. This iminophosphorane shows a high reactivity toward aldehydes and facilitates the formation of the corresponding imine by the aza-Wittig reaction (see Scheme 3).[26] This strategy, which involves additional steps compared to direct RA, has occasionally been carried out on dialkoxybenzaldehyde linkers.

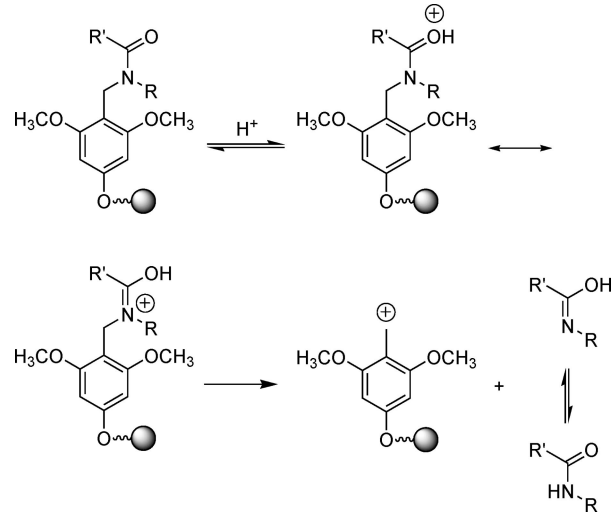
1.4.2. Acylation of Secondary Amines

Acylation of the sterically hindered secondary amine requires attention to the acylating agent and the solvent. For the acylation of trialkoxybenzylamines, preformed symmetrical anhydrides or activation with HATU is preferred; in our own research, symmetrical anhydrides have served well over a broad range of applications. Preferred reaction medias are DCM or a DCM−DMF (9:1) mixture, while the reaction often runs poorly in neat DMF. While the reaction typically is performed at ambient, microwave heating at 60 °C can significantly reduce the reaction times.[25] In contrast to the secondary dialkylamines, BAL-anchored anilines are difficult to acylate.

Scheme 3. Application of Iminophosphoranes As Oxophilic Nucleophiles for the Formation of Imines from Aldehydes



Scheme 4. Proposed Mechanism for Acidolytic Release of Amides with Imidate Intermediate: R = −H (PAL), −CHR′COR″ (BAL)[27]



1.4.3. Release of Final Products: Mechanistic Aspects

All BAL-type handles, except for one so far, rely on acidolysis, i.e., the treatment with an acid, for the release (cleavage) of the final peptide from the support. The most commonly used acids are TFA, HF, and trifluoromethanesulfonic acid (TFMSA). Acidolytic release of a peptide from an amide linker is likely to commence with protonation of the amide carbonyl oxygen, generating an imidate intermediate (Scheme 4).[27] Cleavage of the nitrogen-handle linkage releases the imidate, which tautomerizes to the corresponding amide.

Both BAL and C-terminal PAL anchoring generate the same trialkoxybenzyl carbenium ion upon acidolytic release of the peptide (Scheme 4). However, whereas the PAL handle releases peptides as their C-terminal primary amide, the BAL handle releases a secondary amide in the peptide backbone. Albericio and Barany have reported that the PAL handle required concentrated TFA for its release; they summarize the requirement as 70−90% (v/v) TFA in DCM for efficient release of peptides, whereas lower concentrations of TFA in DCM (5−50%) only gave partial release.[15] In contrast

hereto, the structurally analogous trialkoxybenzyl BAL handles can release peptides at significantly lower TFA concentrations (as little as 1% TFA in DCM). The higher acid-lability of peptidyl−BAL linkages, compared to peptidyl−PAL, must be due to differences in the leaving group, i.e., a better leaving group capability of *N*-substituted amide versus a nonsubstituted amide.

Explorations with molecular mechanics of acidolytic release of model peptides from a model linker using an isodesmic pseudoequilibrium strongly indicated ground-state destabilization of peptides anchored to a BAL handle due to steric factors.[27] Release of the peptide thus involves steric relief, which makes the release energetically more favorable. Therefore, stability of the carbenium ion formed from the linker alone is not sufficient for evaluating handles. On the basis of these molecular mechanics investigations, previous experimental findings on the higher acid-lability of BAL versus PAL anchoring were rationalized. The steric cost of BAL versus PAL anchoring was calculated to 17 kJ/mol for the Gly analogue and 30 kJ/mol for the Ala analogue. Both of these numbers are positive, indicating that the ground-state destabilization is an important factor in release of amides from BAL handles. It is also clear that the presence of the side chain in the Ala model introduces a significant destabilization compared to the sterically less crowded Gly model. The finding that steric crowding leads to increased acid-lability can be important for development and use of linkers, also beyond linkers with substituted benzyl cores and linkers that release amides.

1.4.4. Monitoring On-Resin Reactions

Monitoring the efficiency of the RA and the acylation of the BAL-anchored secondary amine is important for the optimization of protocols and the control of routine procedures. The operationally most simple approach is to quantify the Fmoc group introduced in the acylation step, i.e., at the dipeptide stage. While this often is sufficient, more direct alternatives are sometimes required.

The incorporation of an internal reference amino acid (IRAA) between the support and the linker enables, after acid hydrolysis of the peptidyl-resin, quantification of the amount of incorporated amino acid relative to the IRAA.[28] This relies on chromatographic amino acid analysis and was also applied in the original BAL paper.

Protocols for simple, robust, and preferably nondestructive quantification of aldehyde functional groups on-resin would be quite useful. Three approaches to quantification of the aldehyde moiety of trialkoxybenzyl BAL handles have been described. Vazques and Albericio described a color test based on *p*-anisaldehyde in the presence of sulfuric acid and acetic acid.[29] Shannon and Barany used hydrazone formation in both of their approaches.[30] The first relied on the reaction of resin-bound aldehydes with the well-established reagent 2,4-dinitrophenylhydrazine. The presence of aldehydes was detected by formation of a red to dark-orange color. For the second method, they prepared 4-(9-fluorenylmethyloxycarbonyl)phenylhydrazine (FmPH), which was then reacted with support-bound benzaldehydes to form hydrazones. After removal of excess reagent, treatment with piperidine−DMF gave a dibenzofulvene−piperidine adduct, which could be precisely quantified by a protocol akin to the one used for Fmoc quantification. All three methods are destructive in practice and use small amounts of resin.

A direct strategy for measuring of reactions on the trialkoxybenzyl BAL handle was provided by Albericio and co-workers by introduction of a ^{13}C label at the aldehyde moiety, which was used to monitor reactions leading to BAL−carbamate formation.[31] Ley and co-workers have also prepared PALdehyde, which was ^{13}C-enriched at the carbonyl (using lithiation with *n*-BuLi). It was mixed with commercial resins and used to follow Bu_4NBH_4 mediated RAs by magic angle spinning (MAS) NMR, as the labeled carbon underwent a large change in chemical shift from about 185 to 41 ppm.[32]

2. Synthesis and Design of Backbone Amide Linkers

2.1. BAL with a Benzyl Core

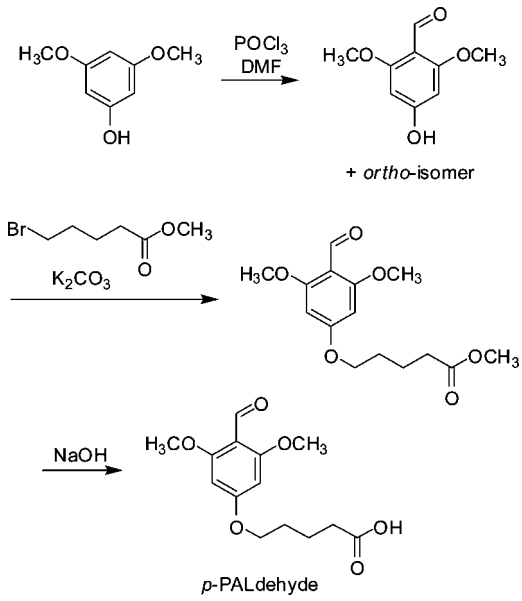
2.1.1. Trialkoxybenzyl BAL

As the first BAL based on the trialkoxybenzaldehyde core structure created an interest in this strategy, a need for efficient synthesis of BAL handles and new chemical methods utilizing these linkers evolved. For example, synthesis of the *para*-isomer of the trialkoxybenzaldehyde BAL linker was somewhat cumbersome due to formation of several byproducts during the formylation process, or the requirement of expensive starting material. To circumvent these problems in the synthesis, we aimed for the *ortho*-BAL isomer, in which the spacer moiety is introduced at a position *ortho* to the formyl group. The problem was also addressed by other synthetic approaches. The demand for linkers with different acid-labilities led to the development of several new BAL handles based on both homo- and heteroaromatic core structures.

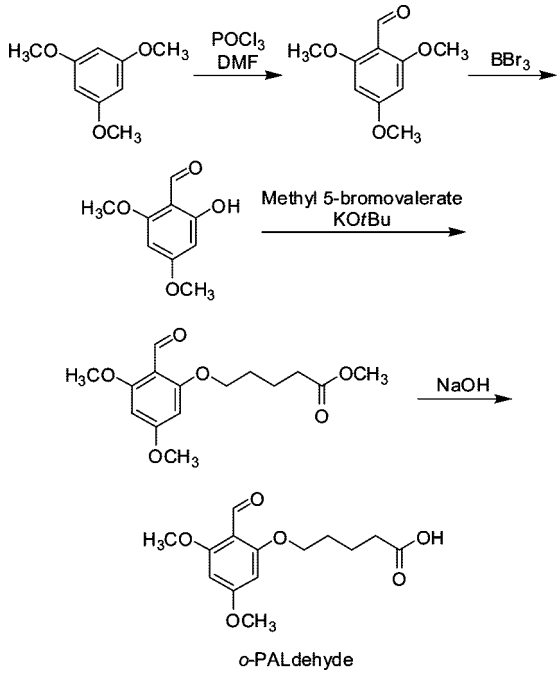
A linker reported by Ellman's group for the synthesis of libraries of 1,4-benzodiazepine-2,5-diones was based on a trialkoxybenzaldehyde core structure, which was attached directly to the solid phase by alkylation of chloro- or bromomethylated PS.[33] Simultanously and independently, a similar linker design for peptide synthesis was developed in Barany's group.[6] This design introduced a five-carbon spacer between the benzene core and the solid phase, which was an important factor for improving the yields of the released substrates.[23] Synthesis of the original *para*-BAL reported by Jensen et al. relied on a synthetic sequence developed by Albericio and Barany for preparation of the key aldehyde (see Scheme 5).[15] Here, 3,5-dimethoxyphenol was formylated by Vilsmeier−Haack formylation; however, two monoformylated isomers were obtained together with a diformylated product, which were separated by recrystallization. Hence, the overall procedure was somewhat inconvenient. A report by Lin and co-workers improved the initial procedure by performing the formylation reaction at low temperature with slow addition of DMF providing a mixture enriched with the *p*-formyl phenolic isomer.[34] Trituration from chloroform provided the pure isomer in 56% yield. The spacer was subsequently introduced by Williamson ether formation in the presence of either potassium carbonate or potassium *tert*-butoxide, as before. Cleavage of the methyl ester was either performed in a one-pot reaction or as a separate reaction. The phenolic intermediate has also been prepared by a lithiation protocol.[35]

In order to circumvent the modest *para*-selectivity in the formylation step, a directed synthesis of the *ortho*-isomer of PALdehyde was pursued. The *o*-BAL handle was already

Scheme 5. Original Synthesis of *p*-PALdehyde by Albericio and Barany[15]



Scheme 6. Regioselective Synthesis of *o*-PALdehyde[36]



implicit in the original BAL paper[6] but first explicitly studied by Boas et al.[36] Because the phenol precursor has an aromatic formyl group *ortho* to the hydroxyl, this intermediate can be accessed by a neighboring-group directed, selective demethylation. The synthetic sequence started from inexpensive symmetric trimethoxybenzene, which was formylated, followed by regioselective demethylation (desymmetrization) of one of the *ortho*-alkoxy groups on the trialkoxy-substituted benzaldehyde using boron tribromide or aluminum chloride (see Scheme 6). The selectivity in the demethylation step was directed by the adjacent formyl group, which served as O-donor to the oxophilic demethylation reagent. The preferred reagent was BBr_3, which was used either neat at −60 °C or more conveniently as a 1 M solution in DCM at 4 °C. Thus, an *ortho*-BAL handle very similar to the originally described *para*-BAL was synthesized in a few selective steps from inexpensive starting material.[36]

2.1.2. Mono- and Dialkoxybenzyl BAL

Less acid-sensitive aldehyde linker designs based on 4-alkoxybenzyl and 2,4-dialkoxybenzyl core structures, respectively, have been prepared by several approaches and were reported by several research groups in the late 1990s. These reports mainly focused on the synthesis of small organic compounds through a "traceless" anchoring. Whereas the acid-labilities of these less-substituted structures are lower than those of the trialkoxybenzyl systems, the less-substituted structures might benefit from less steric hindrance, which possibly could favor some of the chemical transformations taking place at the linker during solid-phase synthesis.

In an early report, Fivush and Wilson utilized a straightforward strategy for the synthesis of an acid-labile dialkoxy-BAL linker (AMEBA = acid-sensitive methoxybenzaldehyde) by the on-resin Swern oxidation of the benzylic alcohol in the commercially available Sasrin (3-methoxy-4-hydroxymethylphenol) linker to the corresponding aldehyde.[8] Likewise, oxidation of a Wang resin gave a monoalkoxy-BAL. However, the strategy may be unfavorable because of incomplete oxidation, which may lead to heterogeneous loading of the resin. After RA of the AMEBA using 2 equiv of primary amine and 2 equiv of $NaBH(OAc)_3$, the solid-phase-bound secondary amine was reacted with a variety of electrophilic "capping reagents" including acid chlorides, chloroformates, sulfonyl chlorides, and isocyanates to afford solid-phase-bound amides, carbamates, sulfonamides, and ureas, respectively. These substrates could be released from the resin by treatment with TFA−DCM (1:19), although aniline amides required treatment with TFA−DCM (1:1).

An alternative for the synthesis of mono- and dialkoxybenzaldehyde linker motif was reported by Swayze, who attached the linker structures by Mitsunobu etherification to ArgoGel resin.[9] The monoalkoxybenzaldehyde linker resin could be converted to the corresponding secondary alkyl- and arylbenzylamine by RA with BH_3-pyridine applying as little as 1.15 equiv of primary amine compound (in a two-step procedure starting with overnight formation of the imine). In this short report, the secondary amine was reacted with a variety of electrophiles, active esters, isocyanates, and sulfonyl chlorides providing solid-phase-bound amides, ureas, and sulfonamides, respectively. Acylation of the secondary amine could take place under standard active ester formation with HATU in a DCM−NMP (1:1) mixture. Swayze stated that, by using less-substituted benzene derivatives, acylation of the secondary amine was facilitated. As expected, these linkers possess a lower acid-lability due to the lower degree of alkoxy substitution. Exposing the functionalized resin to TFA−Et_3SiH (97.5:2.5) for 6−18 h released the secondary amide substrates from the resin, whereas the sulfonamide and urea substrates were cleaved significantly faster from the resin (0.25 h) because of the better leaving group character of these compounds.

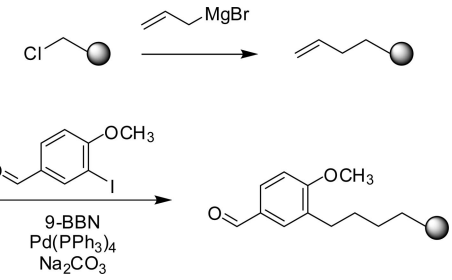
In a short report on a 2,4-dialkoxybenzaldehyde linker, by Sarantakis and Bicksler, 2-methoxy-4-hydroxybenzaldehyde was anchored to chloromethylated PS resin using Williamson ether synthesis conditions.[10] RA of the aldehyde was carried out in a two-step sequence with TMOF as the dehydrating agent, with the amount of formed imine measured by solid-phase IR measurements. Reduction of the imine was performed with $NaBH_4$. Various acylation conditions such as Fmoc-amino acid chlorides, HOBt esters, or symmetric anhydrides, were applied. In contrast to the reports by Fivush and Swayze, Sarantakis and Bicksler investigated

the use of the dialkoxybenzaldehyde system for the synthesis of C-terminally modified peptides by subsequent peptide chain elongation under standard Fmoc-conditions. Interestingly, the authors state that the release of the peptide substrates from this system, which was quite similar to the system reported by Swayze, could be performed with TFA−DCM (3:7) for 10 min.

These BAL analogues have found wide use as inexpensive BAL handles because of the ease of linker synthesis by a one-step procedure from commercially available alkoxyhydroxybenzaldehydes. Especially the dialkoxy core structures have found use in Fmoc SPPS of cyclic and C-terminally modified peptides such as C-terminal ketones[37] (further discussed under section 3.3.3) or azides[38] for segment coupling. The dialkoxybenzaldehyde linker has been further applied as a TFA-labile amide linker for a three-component condensation of benzaldehyde, aniline, and cyclopentadiene providing tetrahydroquinolines on solid phase.[39] Even though HF has been the method of choice for the release of substrates from the monoalkoxybenzaldehyde linker, strongly protic acids may serve as less-hazardous alternatives to HF. Virta and Lönnberg applied the monoalkoxybenzaldehyde linker for the solid-phase synthesis of macrocyclic cryptant-like peptides and released the substrates from the resin by utilizing a mixture of HBr, AcOH, and TFA mixed with anisole as a scavenger (synthesis further discussed under section 3.2.4).[40] Brandt et al. demonstrated release of a pentapeptide in good yield from a monoalkoxy BAL by a 2 h treatment with TFMSA−TFA (1:9) at room temperature.[41]

In an alternative design of a slightly more acid-labile monoalkoxybenzyl BAL, Gu and Silverman introduced the alkyl spacer directly to the aromatic core of 4-methoxybenzaldehyde via carbon−carbon formation.[42] Introduction of the spacer by a Suzuki reaction between vinyl-modified PS resin and 3-iodo-4-methoxybenzaldehyde gave a linker that released the substrates from the solid phase in refluxing TFA. Introduction of the first amino acid proceeded under standard RA conditions, applying the mild reductant $NaBH(OAc)_3$. The following acylation was performed with HATU as the coupling agent. The peptides remained anchored when exposed to TFA at room temperature, i.e., under conditions for removal of Boc protecting groups, but were released with refluxing TFA. However, use of refluxing TFA for release of peptides may, in some cases, not be operationally convenient. The slightly increased acid-lability of this linker is a consequence of the weakly σ-electron donating alkyl (spacer) substituent *meta* to the formyl group, leading to an aromatic system that is slightly more stabilized during the acidolytic formation of an electron-deficient cationic transition state. In the same study, the authors prepared a 4-methoxy-3-alkylbenzaldehyde linker that contained a double bond in the spacer moiety. The linkers had comparable efficiency in the synthesis and release of dipeptide substrates, albeit higher substrate loadings could be achieved with the "saturated alkyl spacer" BAL (see Scheme 7).

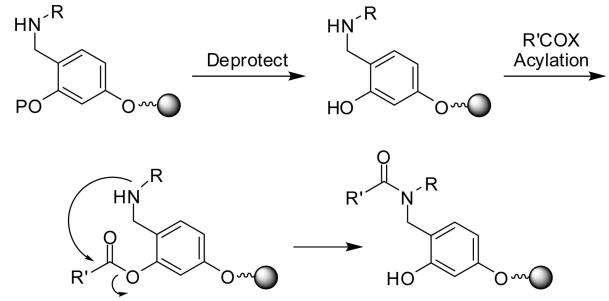
Scheme 7. Synthesis of a BAL Motif for Boc-SPPS by Gu and Silverman[42]



2.1.3. Hydroxyalkoxy BAL and Allyloxyalkoxy BAL: Improving N-Acylation

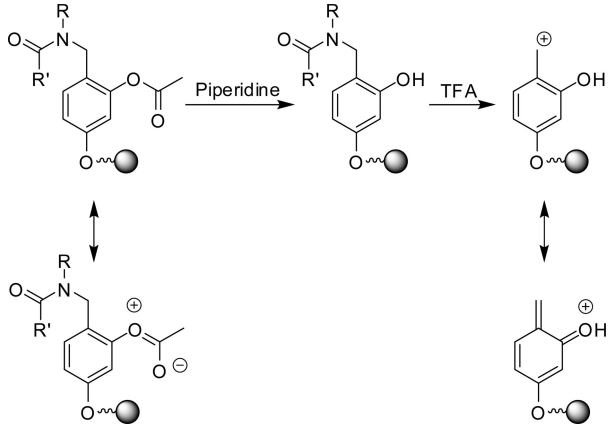
Besides the RA, a key step in formation of the backbone amide linkage is the subsequent acylation step. The acylation step can be dependent on the amino acid side-chain steric properties[6] and the degree of substitution on the aromatic core of the linker. An obvious way to enhance the yield of this step, in cases where it is problematic, would be to increase the electrophilicity of the acylation reagent. However, this strategy may not be a feasible route due to the risk of increased racemization at the α-position, which becomes increasingly acidic with increased "C-terminal" electrophilicity. A potential solution to problematic acylations has been provided by Liley et al.[43] In their strategy, the benzaldehyde linker had an adjacent *ortho*-phenolic hydroxyl group, which the authors suggest acts as a "neighbor group assisting nucleophile" during the acylation process, by intermediate formation of a phenyl ester. If so, then the aromatic core of the linker would serve as a scaffold that keeps the benzylic amine of the first amino acid building block and the phenylester of the second amino acid building block in close proximity, thus favoring reaction to the thermodynamically more stable backbone amide bond (Scheme 8). The method proved effective for introduction of some sterically hindered amino acids like α-aminoisobutyric acid (Aib).

Scheme 8. Acylation by Intramolecular O−N Acyl Transfer from the Initially Formed Phenylester: P = protective group.



As the yields of the initial imine formation were reduced by the presence of the nearby free *ortho*-hydroxy group, this hydroxyl group was transiently protected with an Alloc group creating an allyloxyalkoxy BAL (ALOBAL). After RA, the Alloc group was removed and the subsequent acylation took place in high yield by the O−N acyl transfer strategy. The synthesis of this linker (OHBAL) started out from 2,4-dihydroxybenzaldehyde, which was alkylated in acceptable yield at the 4-position using methyl 5-bromovalerate in the presence of KF following the procedure published by Mendelson et al.[44] Subsequently, the methyl ester was hydrolyzed in good yield by LiOH in THF at room temperature. To monitor the efficiency of the acylation process, the OHBAL linker was attached to the solid phase via a Sieber linker, which allowed the release of the entire substrate−BAL adduct by treatment with TFA−DCM (1:99), i.e., milder conditions than required for the release of the amide substrate from the OHBAL. Several small molecules comprising two building blocks were synthesized in this study.

Scheme 9. Safety-Catch Acetoxyalkoxybenzyl BAL Handle, which in the TFA Stable Mode Is Compatible with Boc SPPS; Upon Cleavage of the Acetoxy Group, The Backbone Amide Linkage Becomes TFA-Labile[45]



2.1.4. Acetoxyalkoxy BAL: Safety-Catch BAL with Reduced Acid-Lability

High acid-lability is often desirable in solid-phase synthesis in order to preserve the structural integrity of sensitive substrates during the acidolytic release from the resin or to retain acid-labile protecting groups. However, in some cases, the ability to remove TFA-labile *t*-Bu-based protective groups is desirable prior to further transformation of the solid-phase bound substrate.

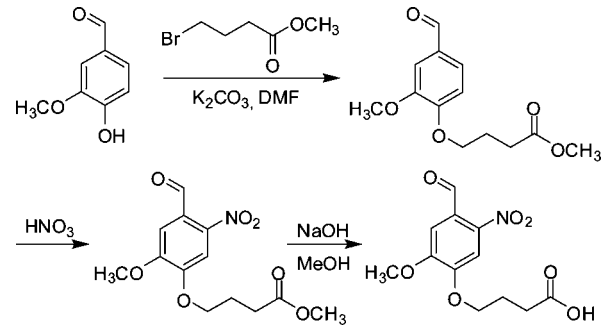
A strategy for lowering the acid-lability during solid-phase transformations was reported by Hruby and co-workers, who developed a BAL based on a 4-alkoxy-2-hydroxybenzaldehyde core structure (AHB-linker).[45] In this structure, the 2-hydroxy group was acetylated prior to synthesis, thereby reducing its ability for electron donation. During peptide chain assembly, this linker had a relatively low acid-lability, which permitted removal of transient *t*-Bu-based protective groups. Prior to release of the final peptide, the *o*-acetyl was removed with piperidine−DMF (1:4) to provide the corresponding AHB BAL, which was labile to treatment with TFA−H_2O (19:1). The linker was synthesized from 2,4-dihydroxybenzaldehyde in a procedure analogous to the procedure reported by Liley et al.[43]

In this strategy, following peptide chain assembly, a piperidine treatment cleaves the phenyl ester to liberate the *ortho*-hydroxyl group. Already in this early report, the authors mention the possibility to accelerate acylation of the secondary amine by a neighbor group effect, resulting in O−N acyl transfer from the adjacent free hydroxyl group. However, this possibility was not investigated. The linker was used in the synthesis of C-terminally modified amino acids and amino acid hydrazides, which were less prone to release under TFA conditions but could be released by HF (synthesis further discussed under section 3.3.3; see Scheme 9).

2.1.5. Deactivated BAL, BAL Mimics

An electron-deficient benzyl BAL with a *para*-carboxy-substituted benzaldehyde core structure was used to investigate the efficiency in the formation of spiroimidazolidinone substrates on solid-phase SynPhase lanterns.[46] In this study, the entire molecular structure of the substrate attached to the BAL mimetic was cleaved from the resin after synthesis, and the purity was investigated as a measure for the anchoring reaction efficiency. This strategy was similar to the "entire release strategy" reported by, e.g., Liley et al.,[43] albeit the present system was much more tolerant toward even strong acidic conditions.

Scheme 10. Synthesis of a Photolabile BAL by Minkwitz and Meldal[47]



2.1.6. Photocleavable BAL

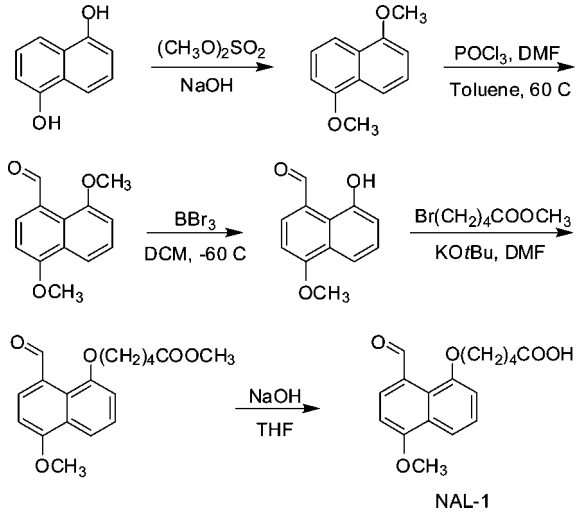
An amide linker that released peptidic substrates from the solid phase by exposure to light has been reported by Minkwitz and Meldal.[47] The linker was based on a nitro-substituted alkoxybenzaldehyde core, and the substrate was released orthogonally to both acid- and base-labile protecting groups by long-wave (365 nm) UV light (Scheme 10). The linker was synthesized from 3-hydroxy-4-methoxybenzaldehyde, which was *o*-alkylated by methyl 4-bromobutyrate. The resulting benzaldehyde was nitrated by treatment with cold concentrated nitric acid, and subsequently the methyl ester was hydrolyzed by NaOH. Finally, the linker and an internal reference, 3-phenylpropionic acid, were bound to a base-labile HMBA (4-hydroxymethylbenzoic acid) PEGA resin, and the cleavage efficiencies were monitored by high-performance liquid chromatography (HPLC).

The presence of a nitro moiety at the aromatic core unfortunately greatly reduced the nucleophilicity of the secondary amine produced by RA on the precursor aldehyde. The secondary amine was only reactive toward strong acylation reagents like acid chlorides. Even acyl fluorides, anhydrides, and active esters did not give any significant acylation. Although the authors introduced an additional alkoxy group on the benzene ring, this did not provide any significant enhancement of the reactivity of the secondary amine. While the original trialkoxybenzyl BAL handle requires strong acylating conditions to acylate,[6] the secondary amine in this photolabile BAL requires even more forceful and limited conditions. The photolabile linker was applied in the synthesis of nonoligomeric piperazine substrates.

2.2. BAL with a Naphthyl Core

By far most acid-lable linkers have relied on benzyl derivatives as core structures. A potential strategy to increase the carbenium ion stability and, hence, the acid-lability would be to use the increased aromatic dimension of naphthalenes, as it would lead to a lower positive charge density. To investigate this possibility, our research groups designed a series of BAL linkers based on various alkoxynaphthalene core structures. The acronym for these naphthalene amide linkers became NAL.

Scheme 11. Synthesis of the 4,8-Dialkoxynaphthaldehyde-Based NAL-1[48]



2.2.1. Dialkoxynaphthalene BAL

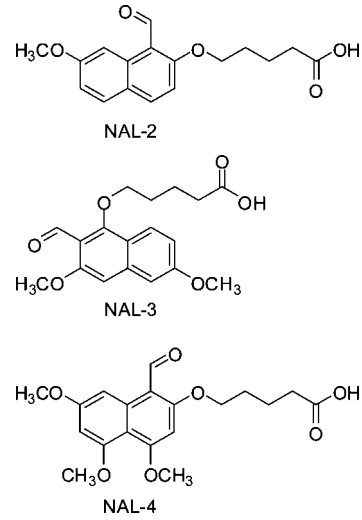
In the initial naphthalene-based linkers, two alkoxy substituents provided additional stabilization of the carbenium ion.[48]

4-Methoxy-8-alkoxynaphthaldehyde Core Structure (NAL-1). In the 4,8-dialkoxy substitution pattern of NAL-1, the 4-position (*para*-position) alkoxy group should stabilize the carbenium ion by resonance, whereas the 8-position (*peri*-position) alkoxy group was thought to possibly stabilize the nearby carbenium ion through space, due to the close spatial proximity.[49] Synthesis of NAL-1 started from easily accessible 1,5-dihydroxynaphthalene, which was methylated by $(CH_3O)_2SO_2$ under basic conditions. Subsequently, the formyl group was introduced by Vilsmeier−Haack formylation. Next, the close proximity of the *peri*-alkoxy group was used to regioselectively demethylate this methoxy group with BBr_3.[50] In this case, the boron atom partakes in a seven-membered ring during the transfer of bromide to the CH_3 group. Analogous to previous BAL handles, a pentanoic acid spacer was introduced to the free phenolic hydroxyl group by treatment with methyl 5-bromopentanoate in the presence of potassium *tert*-butoxide. The spacer ester moiety was cleaved by aqueous sodium or lithium hydroxide (Scheme 11).

2-Alkoxy-7-methoxynaphthaldehyde Core Structure (NAL-2).[48] In NAL-2, the 2,7-alkoxy substitution pattern should provide stabilization of the carbenium ion solely through resonance stabilization (π-stabilization). Also, in the synthesis of this and other naphthalene-based BAL linker designs, the ability to selectively demethylate a methoxy group adjacent to the formyl group proved to be an important feature in the synthetic sequence. The synthesis started with 2,7-hydroxynaphthalene, which was methylated by iodomethane in DMF at 50 °C. This modified methylation procedure gave higher yields and easier workup compared to the original procedure published by Mizutani and coworkers.[51] Demethylation of the *ortho*-methoxy group was carried out with either BBr_3 or $AlCl_3$ as electrophilic demethylation reagents. The spacer was introduced and the ester moiety was subsequently cleaved under standard conditions.

Following RA, acylation of the secondary NAL anchored amine relied on symmetric anhydrides in DCM−DMF (9:1), which was superior to, e.g., active esters. This could indicate that the sterical environment on the naphthalene may be similar to that of the trialkoxybenzaldehyde linkers.

Chart 1. Structures of the dialkoxynaphthaldehyde-based NAL-2,[48] the trialkoxynaphthalene based NAL-3,[52] and the tetraalkoxysubstituted NAL-4.[54]



These NAL-1 and NAL-2 handles showed a somewhat lower acid-lability in comparison to the trialkoxybenzyl BAL. However, NAL-2 showed a higher acid-lability than the dialkoxybenzaldehyde linkers. The NAL-1 and NAL-2 handles were applied in the synthesis of Leu-enkephalin and C-terminally modified Leu-enkephalin, which were released in high yields and high purities.

2.2.2. Trialkoxynaphthalene BAL

To achieve a greater stabilization of the bicyclic aromatic carbenium ion, three alkoxy groups were introduced to the naphthalene core (Chart 1).[52] The 1-, 3-, and 6-positions of the alkoxy groups should provide maximum stabilization of the carbenium ion by resonance stabilization. In contrast to NAL-1 and NAL-2, the synthesis of NAL-3 did not start from a naphthalene derivative but from a benzene derivative, 3-phenoxyacetic acid, which was converted to the corresponding acid chloride and reacted with a malonic ester. The acylated malonic ester was ring-closed by an intramolecular Friedel−Craft reaction, followed by aromatization to provide the naphthyl system in good yield. After methylation of the free hydroxyl residues by iodomethane, the methyl ester was reduced to the corresponding alcohol by $LiAlH_4$. The alcohol was reoxidized to the aldehyde by pyridinium dichromate. Like in the synthetic design of the former benzyl- and naphthyl-based linkers, the aldehyde functionality was utilized as a directing group for the subsequent demethylation of one of neighboring methoxy group with BBr_3 at low temperature. Interestingly, the 1-hydroxy regioisomer was predominantly formed during this step (86%), whereas the 3-hydroxy isomer was formed in only 1% yield. Investigation of the trialkoxy compound by X-ray analysis indicated that this was caused by "out-of-plane" distortion of one methoxy group as a consequence of steric crowding. This crowding is a direct consequence of the close proximity of the *peri*-substituents creating an excessively crowded environment between the formyl group and its *peri*- and *ortho*-substituents. The 1-methoxy group is more prone to demethylation due to steric relief during formation of the less-crowded demethylated product.[52] In addition, the steric crowding forced the formyl group out of plane with the aromatic system. As

we shall see later, the "out-of-plane" twisting of the formyl substituent could indicate that an eventual BAL linkage would have lower acid-lability. Subsequent to the demethylation, the spacer was attached via the free hydroxyl group under standard conditions.

The NAL-3 linker was attached to an aminomethylated PS resin by PyBOP-mediated amide formation and applied in initial solid-phase synthesis of a dipeptide in a synthetic sequence comprising two basic steps. Subsequent cleavage studies showed a significantly lower acid-lability of this naphthalene linker in comparison to its dialkoxy precedents. The dipeptide was released in only moderate yields (only 13% released peptide after 1 h of treatment with TFA−DCM (19:1) and 56% after 2 days of exposure to TFA−DCM (19:1)), thus providing an acid-lability comparable to that of the monoalkoxybenzyl linkers. The time-dependent increase of peptide cleavage from the resin, on the other hand, strongly indicates that the low yield is due to poor ability to release the substrate from the resin and is not a consequence of ineffective RA or acylation.

2.2.3. Tetraalkoxynaphthalene BAL

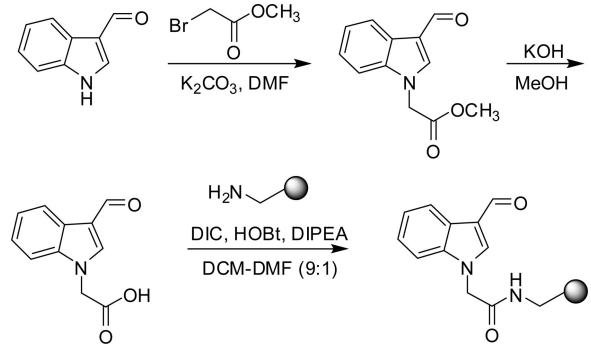
By achieving resonance stabilization from four alkoxy substituents together with low steric crowding, the NAL-4 design was expected to have higher acid-lability in comparison to its precedents (Chart 1). In view of the importance of the optimal substituent pattern on naphthalene to provide the maximal carbenium stabilization, the 2,4,5,7-tetraalkoxynaphthaldehyde substitution pattern was predicted by computational studies to provide low steric strain and high resonance stabilization.[53]

The NAL-4 handle was synthesized in six steps from chromotropic acid (4,5-dihydroxy-2,7-naphthalenedisulfonic acid).[54] The chromotropic acid was converted to the corresponding 2,4,5,7-tetrahydroxynaphthalene in good yield by an alkali melting procedure.[55] The hydroxynaphthalene was methylated under standard conditions by $(CH_3O)_2SO_2$ in the presence of base and subsequently subjected to Vilsmeier−Haack formylation. The very mild conditions used for formylation, a reaction temperature of −5 °C, indicated a high electron-richness (nucleophilicity) of this tetraalkoxy-substituted system. Regioselective *ortho*-demethylation occurred in good yield under standard conditions with BBr_3. After introduction of a spacer and subsequent ester hydrolysis in excellent yields, the linker was attached to aminomethylated PS resin by PyBOP-mediated amide bond formation. The linker was tested by release of a dipeptide from the resin, which revealed a very high acid-lability, releasing a *t*-Bu-protected dipeptide in 63% yield from the solid phase by treatment with 0.5% TFA in DCM for 2 h. To investigate the ability to release fully protected peptides, Fmoc-Tyr(*t*-Bu)-Gly-Gly-Phe-Leu-O*t*-Bu (protected Leu-enkephalin) was assembled on the solid phase and released while retaining acid-labile *tert*-butyl ether and ester protective groups. However, a disadvantage of this linker is its lengthy and nontrivial synthesis.

2.3. BAL with a Heteroaryl Core

An alternative way to achieve increased stability of the carbenium ion is by introduction of heteroatoms capable of stabilizing the carbenium ion by heteroatom lone-pair donation. Generally, heteroaromatic core structures may be divided into basic and neutral heteroaromatics, respectively. Whereas nitrogen-containing heteroaromatics (e.g., pyrroles) are basic heteroaromatics, the corresponding sulfur-containing heteroaromatics (e.g., thiophenes) are neutral.

Scheme 12. Synthesis of an Indole-Based BAL[56]



2.3.1. Indole BAL

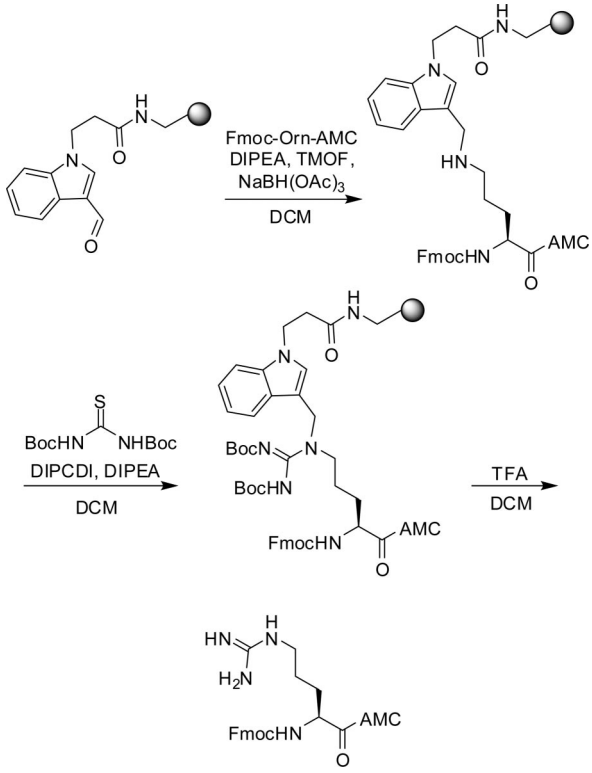
This type of BAL based on an indole core structure was introduced in the late 1990s by Estep and co-workers.[56] The structure was synthesized in a straightforward two-step sequence from 3-formylindole by, first, introduction of a spacer moiety by *N*-alkylation of the indole system with methyl bromoacetate, followed by hydrolysis of the ester in good yield (Scheme 12). The alkylation forms a tertiary amine as a carbenium ion stabilizing heteroatom. The use of inexpensive starting materials and its facile synthesis makes this an attractive linker. The indole linker has, in some cases, an acid-lability (complete substrate release with 1% TFA after 8 h) close to that of the trialkoxybenzyl BAL.

In the first report, on-resin RA was performed using first $NaBH(OAc)_3$ and then $NaBH_3CN$ as reducing agents.[56] This sequential use of reductants was reported to minimize impurities and increase yields. Subsequent acylation of the secondary amine was performed in good yields with the peptide coupling reagent PyBrOP, presumably by intermediate formation of the acid bromide or anhydride. A range of electrophiles such as anhydrides, sulfonyl chlorides, chloroformates, isocyanates, and guanidinylation conditions were applied for the generation of solid-phase bound amides, sulfonamides, ureas, carbamates, and guanidines, respectively. The corresponding substrates were released in generally good yields from the linker by a 4 h exposure to TFA−DCM (1:1).

Comparative studies by Yan et al. showed that the indole linker released the different substrates faster than dialkoxybenzaldehyde BAL handles and Rink amide resins.[57] However, a comparison of acid-lability between the indol- and trialkoxybenzyl-based BAL handles was not performed in this interesting study. The various substrates were generally released from the indole resin in the order sulfonamide > carbamate ≈ urea > amide > amine. Furthermore, the authors state that the indol linker is more economical than the dialkoxybenzyl and Rink amide linker structures.

In an alternative anchoring mode described by Beythien, White, and co-workers, the side chain of ornithine was attached by RA to indole BAL.[58] After formation of the secondary amine, the ornithine derivative was converted to the arginine by electrophilic guanidinylation (Scheme 13). This strategy was used in the synthesis of arginine amide methyl coumarins (AMCs) and was reported to be superior to other solid-phase methods, including one relying on Rink amide resin, with on-resin formation of an ornithine side-chain thiourea, which, after peptide-chain elongation, was

Scheme 13. Transformation of Ornithine to Arginine by Side-Chain Indole BAL Anchoring and Guanidinylation[58]



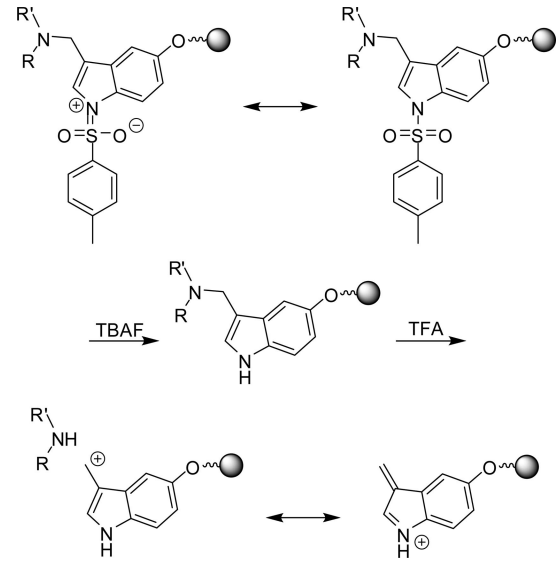
converted to the corresponding side-chain unprotected arginine by *S*-methylation and ammonolysis, followed by release from the resin.

The indole linker has found widespread use in solid-phase organic synthesis, and the indole design has been modulated in different manners to fit specific purposes. Gmeiner and co-workers attached an indole-type linker to the support by a Huisgen 1,3-dipolar cycloaddition between an azide and an alkyne to form a triazole-containing spacer. The linker-resin was used for parallel synthesis of piperazine-containing dopamine-receptor ligands;[59] here, $NaBH(OAc)_3$ was used as a reducing agent. The subsequent acylation was performed by a HOAt-assisted carbodiimide coupling via the active ester to form the corresponding amide on the solid phase (synthesis also mentioned under section 5.2.2). These conditions are somewhat "milder" than what was reported initially by Estep and co-workers. The authors state that the presence of a triazole in the spacer provides more robustness. Furthermore, this linker apparently released the substrates with higher purities and yields compared to the original indole linker. This may be a consequence of a longer spacer, which also in the homoaromatic BAL systems seems to have a significant influence on the cleavage yields.

2.3.2. Safety-Catch Indole BAL

Ley and co-workers have developed a safety-catch variation, in which the acid-lability of the indole linker was reduced by conversion of the indole nitrogen to the corresponding sulfonamide by tosylation.[60] Thus, the electron-donating ability of the nitrogen atom was strongly reduced by the adjacent electron-withdrawing *N*-sulfonyl group. After synthesis of the desired substrate on the solid phase, the tosyl group was removed by, e.g., Bu_4NF (TBAF), and final release of the substrate was accomplished by treatment with dilute TFA.

Scheme 14. Basic Principle in the Safety-Catch Indole Linker[60] [a]



[a] Converting the pyrrol amine to the sulfonamide reduces the electron-donating ability of the nitrogen atom. Upon cleavage of the sulfonamide, the electron-donating ability of the nitrogen atom is regained and the acid-lability is increased.

Because the nitrogen atom in this design was already occupied by the electron-withdrawing tosyl group, anchoring to the spacer was performed via a 5-alkoxy substituent, and the synthesis commenced from 5-benzyloxy-3-formyl indole. Initially, the tosyl group was introduced and the benzyloxy group was cleaved by BCl_3. Then the spacer moiety was introduced under Williamson alkylation conditions with *tert*-butyl bromoacetate, followed by acidic ester cleavage.

The tosylated indole linker was resistant to 2 h of treatment with TFA−DCM (1:1), though after 2 days of exposure to these conditions, the amide substrate was completely released. After removal of the *N*-tosyl moiety, the linker completely released the amide substrate by a 1 h treatment with 1% TFA in DCM. The very acid-labile linker, however, also slowly released the amide substrate simply upon standing. Monitoring of substrate release from the safety-catch indole linker was carried out on a Wang linker resin, by incorporating an UV-active anthryl unit (i) in the spacer unit between the solid phase and the linker and (ii) in the amide substrate to be released (see Scheme 14). By treatment with TFA, both the primary Wang linker and the secondary "activated" (detosylated) indole linker cleave from the solid phase. The extent of release, i.e., the ratio between the linker moiety and the anthryl substrate, was monitored by HPLC at 365 nm (anthryl-specific wavelength), as the ratio between the three possible adducts, i.e., (i) the anthryl-labeled linker with attached substrate, (ii) the anthryl-labeled linker without substrate, and (iii) the anthryl-labeled substrate.

2.3.3. Thiophene BAL

Highly acid-labile BAL handles with carbenium ion stabilization in a heteroaromatic ring have also been developed starting from electron-rich 3,4-ethylenedioxythiophene (EDOT).[61] EDOT, which is produced on a multiton scale, is a widely utilized electron donor for the formation of conducting polymer materials.[62] Because EDOT is a highly reactive and oxidation-sensitive compound, the electron density was first reduced by formylation to introduce an

Scheme 15. Three-Step Synthesis of Thiophene-Based T-BAL3, Introducing the Spacer Moiety via an Exocyclic Sulfur Atom

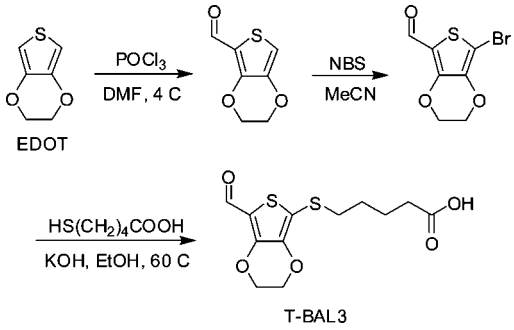
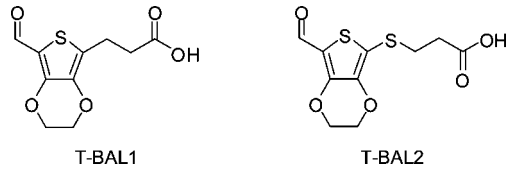


Chart 2. Thiophene-Based Backbone Amide Linkers T-BAL1 and T-BAL2.

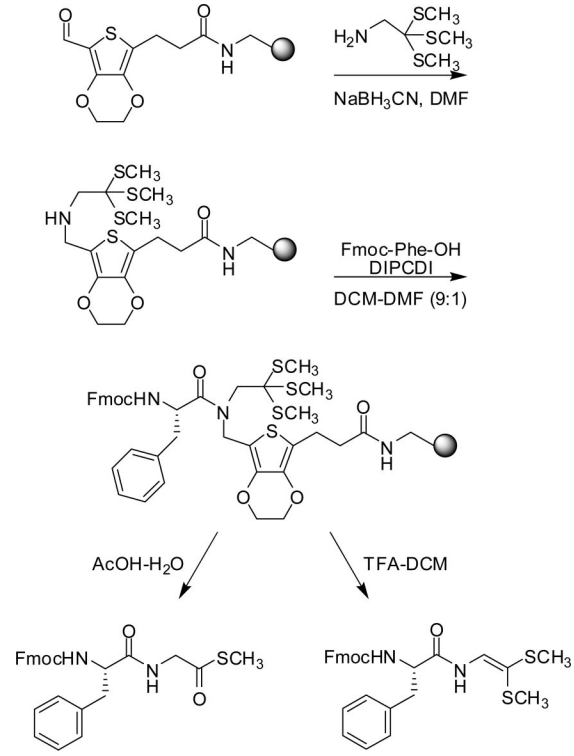


electron-withdrawing aldehyde moiety. The formylated EDOT was iodinated under mild and selective conditions by *N*-iodosuccinimide in dilute acetic acid.[63] In the initial design, the spacer moiety was introduced directly onto the thiophene core via a highly chemoselective carbon−carbon bond formation. By applying a mild Negishi coupling procedure, the carbon−carbon formation took place in the presence of an unprotected formyl group on the thiophene. Because introduction of the spacer moiety was combined with basic hydrolysis of the methyl ester, the linker molecule was obtained in just three overall steps and in good yield.

However, to circumvent the C−C bond-forming step, later designs of this highly acid-labile linker type relied on introduction of the spacer via a thioether bond (T-BAL3). The facile introduction of the spacer was achieved simply by nucleophilic aromatic substitution of the "EDOT−bromide" with a mercaptoalkyl acid. The chemoselectivity of the aromatic-substitution reaction enabled introduction of the spacer without protecting the carboxylic group on the spacer (Scheme 15, Chart 2). Although the introduction of an exocyclic sulfur might be expected to increase the ability to form stable carbenium ions in comparison to the initial T-BAL1 design, this was not observed.

Because of the high electron-richness, a low electrophilic reactivity of the formyl group was expected, and model experiments in solution were performed. These solution-phase experiments (DMF−MeOH mixture) indicated that RA was possible, and a normal *N*,*N*-dialkylation pattern with DMF as solvent was observed; in this respect, T-BAL1 was similar to the trialkoxybenzyl BAL system. However, on-resin RA under standard conditions (amino acid hydrochloride and $NaBH_3CN$ in DMF), which had been successful for homoaromatic BAL handles, generally resulted in low yields. An even lower reactivity of the aldehyde was observed for the thioether-substituted T-BAL3 linker, as only modest yields after even prolonged reaction times and further addition of reagents were obtained. Fortunately, performing the RA under microwave heating to 60 °C resulted in good yields of the corresponding thienyl amines on the T-BAL3 system, and after peptide-chain assembly, the final products were released in good yields by a 2 h treatment with

Scheme 16. Solid-Phase Synthesis of C-Terminal Thioesters and Ketene Dithioacetals Using T-BAL1[a]



[a] The thioester was released under mild aqueous conditions by acetic acid, while the corresponding ketene dithioacetal was released under nonaqueous conditions by TFA.

TFA−DCM (1:19). The X-ray crystal structure of T-BAL2 did provide few explanations in terms of electronic factors.[64]

Generally, microwave heating proved useful for achieving higher yields in the initial amination of the thiophene linkers as well as for the acylation step. First, several dipeptides and the Leu-enkephalin pentapeptide were synthesized in good yields. This new linker design was furthermore applied for the synthesis of C-terminal peptide aldehydes and acetals. T-BAL handles could even be used for solid-phase synthesis of some secondary amines, which were released in moderate yields by hot TFA. Also C-terminal peptide thioesters were synthesized using the trithio-*ortho*-ester (TTO) strategy[65] (described in section 3.3.2), where the sensitive thioester substrates could be released in acceptable yields under very mild conditions by treatment with aqueous acetic acid (Scheme 16). This remarkable and extremely high acid-lability could be explained with ground-state destabilization due to steric hindrance from the TTO ester moiety. Cleavage by acidic nonaqueous conditions resulted in release of the corresponding peptide ketene dithioacetal in good yield (Scheme 16).

3. Applications in Peptide Synthesis

Naturally occurring peptides as well as synthetic analogues include not only linear peptide acids and amides but also numerous examples in which the C-termini are modified. Examples of C-terminal functional groups include alcohols, ethers, esters, *N*-alkylamides, *N*,*N*-dialkylamides, hydrazides, trifluoromethyl ketones, aldehydes, mercaptoalkylamides, thioesters, and thioamides. Similarly, cyclic peptides are both naturally occurring and important synthetic targets. C-Terminally modified peptides are important in enzymology

to study substrate specificity and catalytic mechanisms, to provide sensitive assays, and as inhibitors and analogues with specifically designed activities. Modifications of C-termini, as well as elimination of termini through cyclization, are important tactics in the design of more effective therapeutic agents, as they reduce conformational flexibility and allow alteration of bioavailability by protecting the peptide 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, such as thioesters, are useful as intermediates in convergent synthesis.

The BAL strategy allows for the preparation of peptides with a variety of C-terminal functionalities, as well as a completely general way to prepare cyclic peptides in the solid-phase mode without requiring suitable side-chain functionalities for anchoring.[66] The versatility was demonstrated in the original BAL paper with the synthesis of short peptides with C-terminal alcohol, dimethyl amide, aldehyde, and ester functionalities.[6] For these initial studies, the C-terminal was protected as the *t*-Bu ester (for synthesis of peptide acids) or the allyl ester (for cyclic peptides), or an appropriate C-terminal modified amino acid derivative was used, e.g., an aliphatic ester (for peptide esters), *t*-Bu ether (for peptide alcohols), or dimethyl acetal (for peptide aldehydes).

In a follow-up paper, Barany, Albericio, and co-workers described a way to extend the peptide C-terminally from the BAL-anchored residue.[67] The aim was to access peptides with C-termini labile to nucleophiles (e.g., piperidine used for Fmoc deprotection), exemplified by peptide thioesters and peptide *p*-nitroanilides (pNA). Hence, the peptide could be elongated by standard Fmoc-chemistry, followed by selective removal of the C-terminal allyl ester protection and coupling of preformed amino acid thioesters or pNAs. A potential complication is epimerization of the BAL-anchored residue upon activation via formation of an oxazolonium ion by attack of the BAL−amide oxygen on the activated carbonyl. However, the authors reported that, by addition of solid coupling agent (e.g., HATU or HBTU) as the last reagent, the epimerization was controlled to <2%. The strategy was illustrated with the synthesis of a range of peptide thioesters and pNAs.

In a later approach (2000), the same authors investigated the possibility of extending the C-terminal before peptide-chain assembly in the N-direction.[68] The aim was to enable the synthesis of peptides containing C-terminal Pro or *N*-alkyl amino acids (i.e., with a secondary nitrogen) or C-terminal His (which was found to epimerize in the original BAL strategy). This approach had the advantage that anchoring was through a secondary amine (not acylated to form the amide), and therefore, epimerization through an oxazolonium ion would not happen. Further, as this linkage was stable to TFA, a *t*-Bu ester could be used for C-terminal protection. To demonstrate this, short peptides with C-terminal Pro-OH and Pro-NMe_2 were prepared.

A separate issue is that of diketopiperazine (DKP) formation when using C-terminal allyl ester protection. The original paper[6] described almost quantitative spontaneous DKP formation during Fmoc-removal from the dipeptide, a cyclization presumably favored by a cis-configuration of the BAL-anchored secondary amide. Using Trt- or Ddz-protected amino acids for the first acylation reaction was suggested to avoid DKP formation. These derivatives could be selectively deprotected under weakly acidic condition without BAL cleavage.[69] The third residue was then coupled with in situ neutralization.

Another possibility to avoid DKP formation was reported by Barany and co-workers.[70] It was suggested to perform RA with an allyl ester amino acid and use a standard Fmoc-protected amino acid for coupling to the BAL−amine. Then the C-terminal allyl ester was removed and another C-allyl protected amino acid was coupled, extending the chain in the C-direction. The tripeptide sequence was Fmoc-deprotected with piperidine (without risk of cyclization), and peptide elongation proceeded by standard Fmoc-methodology.

Alternatively, a 1,1-dimethylallyl (DMA) ester protecting group was suggested as the C-terminal protecting group in BAL SPPS.[71] The authors indicate that, because of sterical hindrance, DMA esters would resist nucleophilic attack and, hence, avoid problems with DKP formation. To our knowledge, however, it has not yet experimentally been verified in a BAL synthesis. The DMA group, removed with catalytic Pd(0) and *N*-methylmorpholine, is orthogonal to Fmoc. A series of Fmoc, DMA-ester protected amino acids were prepared in high yields.

3.1. Linear and Branched Peptides

Bourne et al. reported in 1999 how the TFA stability of C−N linkages derived from 4-formylphenoxy linkers can be utilized for the synthesis of linear peptides by Boc-based protocols.[72] In a recent paper, 4-formylphenoxyacetic acid was also used as a linker for assembly of phosphopeptides.[41] These peptides were deprotected on-resin with TFA mixtures. Synthesis on PEGA resins, which swell extensively in aqueous buffer, allowed the authors to display the support-bound peptides to cell lysates and pull down phosphoserine binding 14-3-3 proteins. In total, 15 sequences containing a common phosphorylation motif were prepared and all were able to bind the intended 14-3-3 proteins, whereas similar nonphosphorylated control sequences did not. This BAL strategy allowed variations in the peptide C-terminal.

Virta and co-workers used BAL-anchored pentaerythrityltetraamine ($C(CH_2NH_2)_4$) as the scaffold for assembly of branched peptides.[73] Interestingly, they find that the two methoxy substituents on the linker 4-(4-formyl-3,5-dimethoxyphenoxy)butyric acid are required to avoid cross-linking between two linkers during RA of scaffolds with two unprotected primary amines. With a resin-bound scaffold containing (besides the BAL secondary amine) one free primary amine, one Alloc-protected amine, and the third masked as an azide, the authors were ultimately able to acylate the scaffold with four different amino acids. No acid-labile protecting groups were used, as this dimension was saved for the linker. The strategy was used in the synthesis of 11 pentaerythrityl-branched tetra- and octapeptides.

3.2. Cyclic Peptides

There are comprehensive reviews on the synthesis of cyclic peptides, peptidomimetics, and conjugates;[74] on cyclization of peptides and depsipeptides;[75] on synthesis, conformation, and applications of DKPs;[76] and on combinatorial aspects of DKP and cyclic peptide synthesis, as well as their application as privileged structures.[77]

3.2.1. Diketopiperazines (DKPs)

del Fresno et al. have reported the spontaneous DKP formation of BAL-anchored dipeptide esters.[78] The strategy essentially followed the original BAL publication,[6] suggesting amino acid methyl esters (or their HCl salt) for the RA step. It was emphasized that, with trifunctional amino acids and orthogonal protecting groups, the DKPs anchored to a solid support could serve as useful scaffolds for the introduction of structural diversity. DKPs were synthesized from N^{ε}-Alloc and Mtt protected Lys, while Glu, Asp, and Pro residues also were utilized. Besides the two side chains, alkylation of the non-BAL-linked amide nitrogen was investigated after cyclization to introduce a third point of diversity.

3.2.2. Head-to-tail Monocyclic Peptides

The original BAL paper suggested C-terminal allyl ester protection for synthesis of cyclic peptides.[6] With the recommended precautions to avoid DKP formation, the strategy was illustrated with the successful synthesis of cyclo(Arg-D-Phe-Pro-Glu-Asp-Asn-Tyr-Glu-Ala-Ala). Preferred conditions for on-resin cyclization were PyAOP/HOAt/DIPEA in DCM. Following cleavage from the resin with TFA−Et_3SiH−H_2O (92:5:3), racemization at the point of cyclization (Ala) was evaluated by HPLC. This showed an L/D-ratio of 88:12, with overall purity for both signals >95%.

Barany and Albericio attempted to use the same strategy in the synthesis of circular bovine pancreatic trypsin inhibitor (c-BPTI).[79] Gly 28 in the sequence was chosen as the site of cyclization to avoid problems with epimerization. The linear 58 AA sequence was assembled using standard methodology (the second amino acid was introduced with Ddz-protection), and the allyl and Fmoc groups were removed. A range of conditions for cyclization were studied, but in all cases without success. The desired structure could, however, be prepared by native chemical ligation cyclization in solution after solid-phase synthesis of a peptide thioester using a side-chain anchoring strategy.

Bianco, Guichard, and co-workers used the approach[70] to extend the BAL-anchored dipeptide from the C-terminus to form a C-allyl ester protected tripeptide before removing the N^{α}-Fmoc group in their synthesis of a cyclic hexapeptide.[80] With a sequence consisting of three repetitions of the D-Ala-L-Lys motif, the cyclic peptide formed a template, displaying a CD40L-derived sequence, ^{143}Lys-Gly-Tyr-^{146}Tyr, on the three lysine side chains. The entire structure was assembled on solid phase, with the template being cyclized on-resin with DIPCDI/HOAt before Mtt-deprotection of the lysine side chains and simultaneous assembly of the three chains. The entire structure was obtained in 16% purified yield.

Scytalidamide A, a cyclic heptapeptide found in a marine fungus, was synthesized by Gu and Silverman on their 4-methoxybenzaldehyde linker.[81] After RA with H-Phe-OMe·HCl, the linear heptapeptide was assembled with Boc amino acids. The C-terminal methyl ester was deprotected with LiOH in H_2O−THF (1:7), the N-terminal Boc was deprotected with TFA−DCM (1:1), and on-resin cyclization was accomplished with PyBOP/DIPEA in NMP. The finished Scytalidamide A was released from the linker after refluxing in TFA for 3 h. Overall yield was 46% after purification by flash chromatography. In comparison, 20% isolated yield was obtained after the same synthesis on a phenylalanine silane resin.

Smythe and co-workers used their 4-formylphenoxy linker for the Boc-based synthesis of cyclic heptapeptide Stylostatin 1.[82] RA with amino acid allyl esters (two different cyclization sites were chosen) and acylation of the BAL secondary amines were performed in solution, before the linker−dipeptide structures were anchored to a solid support. Peptide assembly then followed standard Boc SPPS protocols. The allyl ester was removed, and the heptapeptides were cyclized with BOP/2,6-lutidine at −10 °C. HF-cleavage gave Stylostatin in 10−25% yield after HPLC purification.

3.2.3. Cyclic Peptides from β-Amino Acids

Albericio and co-workers have outlined the synthesis of cyclic α,β-tetrapeptides.[83] The synthesized structures hold potential as scaffolds for functionalization as the sequence contained two diaminopropionic acid (Dapa) residues with side-chain amines ready for further functionalization. Hence, c(L-Dapa-βAla-L-Dapa-βAla) was prepared with either N^{α}-Alloc or N^{α}-Boc protection of Dapa. Whereas the former structures could be deprotected selectively for further on-resin functionalization, the latter was deprotected with simultaneous cleavage. Fmoc was chosen as the temporary $N^{\alpha,\beta}$-protecting group, and 4-nitrobenzyl (removed with $SnCl_2$/AcOH/phenol/DMF) was chosen as the C-terminal protecting group. Cyclization was performed with PyAOP/DIPEA and was reported to work better on-resin with the BAL approach, compared to cyclization in solution after synthesis of the linear peptide on a Cl-Trt-resin.

In a 2006 paper, Virta and co-workers further elaborated on BAL synthesis of cyclo-β-tetrapeptides and their potential as scaffolds.[84] The peptides were assembled and cyclized on-resin after removal of the N-terminal (Mtt) and C-terminal (PhiPr) protections. Further selective deprotection of side chains allowed stepwise orthogonal derivatization of the bound templates with various monosaccharide derivatives. A final TFA treatment released the products from the support.

Perhydro-1,4-diazepine-2,5-diones are seven-membered rings, which can be viewed as cyclic dipeptides containing one α- and one β-amino acid. One reported approach to some of these structures begins with RA of trialkoxybenzyl BAL with a β-amino acid ethyl ester, followed by acylation with a Fmoc α-amino acid and subsequent Fmoc-cleavage to obtain the dipeptide ester.[85] The structures were cyclized by treatment with NaOMe in MeOH/NMP and finally released from the linker with TFA−Et_3SiH−H_2O (95:3:2). Conformations were studied with X-ray and NMR, and the authors from Novartis concluded that the rings show rigidity and, hence, could represent scaffolds for receptor ligands. The studies, however, also revealed epimerization of the α-amino acid (the β-amino acid was introduced as a racemate), probably occurring during the cyclization step.

The problem with epimerization was also reported in another systematic study to optimize conditions for (3,7-disubstituted) perhydro-1,4-diazepine-2,5-dione synthesis.[86] Applying a trialkoxybenzyl BAL strategy, the authors used benzyl ester protected β-amino acids for the initial RA. Following acylation with a Fmoc amino acid and Fmoc removal, cyclization proceeded by direct nucleophilic attack of the amine on the ester. Optimal conditions were found to be DBU in dimethyl sulfoxide (DMSO) at 60 °C. 3-Benzylperhydro-1,4-diazepine-2,5-dione, synthesized from β-Ala and Phe residues, was cleaved with TFA−DCM (1:1) in 97% purity.

Smythe and co-workers used 5-(4-formylphenoxy)pentanoic acid as linker for Boc-SPPS of a small 9-member library of cyclic peptides based on the somatostatin sequence.[87] The peptides with the general formula cyclo-[Phe-(D)Trp-AA1-AA2-(βAla)] were assembled by parallel synthesis starting with RA with H-Phe-OAllyl, followed by Boc-SPPS using in situ neutralization and HBTU activation. Allyl deprotection and a final TFA treatment gave the linear peptide on solid phase. Cyclization and cleavage conditions were investigated systematically. BOP/DIPEA in DMF was selected for the cyclization step as this gave low epimerization (of the BAL-anchored C-terminal residue) and low dimer formation. HF was found to give superior yield in the cleavage step, but also HBr/TFA resulted in satisfactory yields over 60−90 min. Altogether, the target peptides were obtained in 68−99% purity and 8−21% yields.

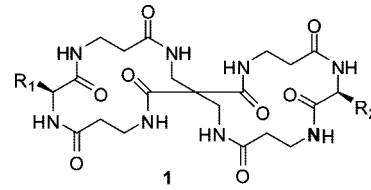
3.2.4. Other Cyclic Peptide Structures

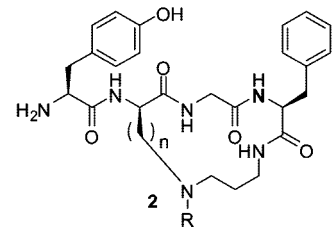
Albericio and co-workers used a DKP scaffold for bicyclic structures displaying a conformationally constrained RGD (Arg-Gly-Asp) motif for binding to integrin receptors.[88] On a trialkoxybenzyl linker, the DKP formed spontaneously after Fmoc-deprotecting Fmoc-Lys(Mtt)-(BAL-resin)Asp(Allyl)-OMe. Next, the Mtt group was selectively removed with TFA−DCM (1:99) and short peptide sequences, containing the RGD motif, were assembled on the Lys side chain of the resin-bound DKP, using standard Fmoc-methodology. After removing the allyl ester on the Asp side chain, the structures were completed by on-resin cyclization with PyAOP. Products were cleaved with TFA−H_2O (9:1) in >75% yield and 50−70% crude purity.

Virta and Lönnberg used synthetic branched building blocks and a double on-resin cyclization strategy to prepare compact cryptand-like bicyclic peptides with a BAL strategy.[40] A relatively acid-stable 4-alkoxybenzaldehyde linker (no methoxy substituents) was chosen. A series of five bicyclic peptides, having two parallel peptide chains and one antiparallel peptide chain, was prepared using Fmoc/Boc chemistry. The first cyclization was effected after removal of Fmoc and allyl ester protecting groups, while the second cyclization followed after Boc and *t*-Bu ester deprotections. Both cyclization reactions were mediated by PyAOP/DIPEA (5/10 equiv, 5 h), and the authors speculated that the achiral nature of their tertiary nitrogen branching units facilitated easy cyclizations. Isolated yields of the five structures were, however, in the range 5−17%.

Virta et al. described the BAL synthesis of β-Ala containing spirobicyclic heptapeptides (**1**, Chart 3).[89] The spiro-branching came from incorporation of orthogonally protected bis(aminomethyl)malonic acid, AllO−CO−C(CH_2NHBoc)-(CH_2NHFmoc)−COOH. Because a racemic mixture of the building block was used, the peptides were obtained as a stereoisomeric pair. A 4-alkoxybenzaldehyde linker was chosen, and the initial RA with H−βAla−O-*t*-Bu was carried out in solution. The authors state that the *t*-Bu group essentially prevented spontaneous cyclization at the dipeptide stage. Peptide assembly hence proceeded by standard Fmoc-chemistry. Both cyclizations were performed with HATU, the first after allyl ester and N-terminal Fmoc deprotection, and the second after *t*-Bu and N-terminal Boc deprotection. Six different structures were prepared, all containing four βAla and two α-amino acids, and all cleaved with HBr−AcOH−TFA (1:3:40) in 10−15% yield (sum of the two diastereomers). Whereas the yield was lower than compared to the synthesis on a Wang resin, with the second cyclization carried out in solution (26% total yield), the BAL approach was preferred as the complete solid-phase synthesis avoided an intermediate HPLC purification before the second cyclization step.

Chart 3. Cyclic peptide constructs from Virta et al. (1)[89] and Goodman et al. (2);[90] the nitrogen in bold indicates the point of anchoring to the BAL handle.





Rew and Goodman synthesized cyclic amine-bridged enkephalin analogues (**2**) on commercial 2-(4-formyl-3-methoxy)phenoxyethyl PS resin.[90] The synthesis pursued the traditional route with RA with H-Phe-OAllyl, followed by acylation with Trt-Gly-OH. 2-Chloro-1-methylpyridinium iodide (CMPI) was used for this difficult coupling. Following Trt-removal with DCM−TFA−*i*-Pr_3SiH (96:2:2), an Fmoc and 2-nitrobenzenesulfonyl (Nosyl) protected 2,3-diaminopropionic acid (alternatively 2,4-diaminobutanoic acid) building block was introduced with HBTU/HOBt under in situ neutralization with 2,6-lutidine in DCM−DMF (1:1). Applying the stronger DIPEA base instead of 2,6-lutidine resulted in epimerization of the building block. Following Fmoc removal, Boc-Tyr(*t*-Bu)-OH was coupled to obtain the tetrapeptide. Next, the sulfonamide side chain was alkylated under Fukuyama−Mitsunobu conditions with *N*-Alloc ethanolamine. The Alloc group and C-terminal allyl ester were removed simultaneously, and cyclization was accomplished with HBTU/HOBt. Finally, the Nosyl group was removed and the bridging amine was alkylated/arylated by RA with a range of aldehydes. The structures were liberated from the linker with TFA−i-Pr_3SiH−H_2O (92:5:3) in excellent purities and yields.

3.3. C-Terminally Modified Peptides

Methods for solid-phase synthesis of C-terminally modified peptides have been comprehensively reviewed by Alsina and Albericio.[91] The topic is also covered in a review on solid-supported synthesis of oligomeric bioconjugates by Virta et al.[92]

3.3.1. Peptide Aldehydes

C-terminal peptide aldehydes are valuable in chemoselective ligation of unprotected peptide fragments, using efficient and highly chemoselective reactions such as oxime[93] and hydrazone[94] formation. Furthemore, their inhibitory property toward many proteolytic enzymes, including HIV protease, is well-described.[95] However, it is challenging to synthesize these compounds on solid phase, and a variety

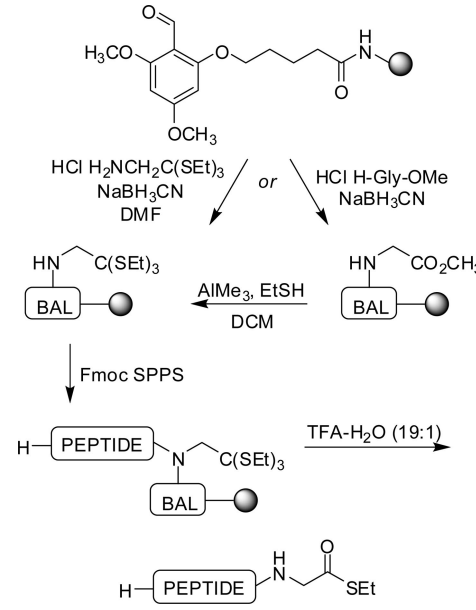
of creative strategies have been proposed, including combinatorial approaches.[96] A few examples are aminolysis of a C-terminally bound peptide with a (masked) amino aldehyde,[97] anchoring by oxazolidine formation between an amino acid aldehyde and resin-bound Ser or Thr,[98] cleavage by ozonolysis of an olefin linker,[99] reduction of a Weinreb amide based linker,[100] or hydrolysis of a semicarbazone linker.[101]

In the original BAL strategy for the synthesis of C-terminal glycinal peptides, aminoacetaldehyde dimethyl acetal was anchored to the linker by RA.[6] After Fmoc-based peptide assembly, treatment with TFA−H_2O (19:1) or other scavenger cocktails deprotected the peptide, demasked the aldehyde from its acetal, and cleaved the product from the linker. This strategy was later used by our group to prepare amphiphilic peptide aldehydes up to 16 residues, which were purified and ligated to aminooxyacetyl-functionalized monosaccharide (pyranoside) templates in solution, to give helix bundle structures referred to as carboproteins.[102]

Jensen, Barany, and co-workers later extended the original strategy to allow peptide aldehydes with C-terminal -Ala-H, -Phe-H, and -Asp-H.[103] The respective amino acetals were synthesized in solution from Z- or Fmoc-protected amino acids via reduction of their Weinreb amides with $LiAlH_4$ or, in the case of Z-Asp(OtBu)-OH via $NaBH_4$ reduction of a mixed anhydride, followed by Swern oxidation of the alcohol. Initially, solution-phase removal of the Fmoc gave disappointing yields; hence, a one-step solid-phase deprotection and RA protocol was developed for these derivatives. Optimal conditions for removal of the Z-group were found to be catalytic transfer hydrogenation with 1,4-cyclohexadiene in the presence of Pearlman's catalyst. After anchoring the amino acetals (1,3-dioxolanes or dimethyl acetals) to the BAL handle, peptide synthesis proceeded following the standard protocol. Treatment of the completed peptidyl-resins with TFA−H_2O (19:1) released the peptide with concomitant hydrolysis of the acetal moiety to free the C-terminal aldehyde. The same result was obtained with reagent R, TFA−thioanisole−ethaneditiol−anisole (90:5:3:2), whereas TFA−ethanedithiol (19:1) resulted in some thioacetal adducts. To address the question of racemization, H-Glu-Val-Val-(L/D)-Phe-H was prepared starting from both Z-Phe-OH and Z-D-Phe-OH. In the L-Phe-H peptide, 10% of the opposite diastereomer (D) was present, while in the D-Phe-H peptide, 26% of the L-form was found.

Barany and co-workers in 2005 suggested an alternative approach for the synthesis of C-terminal peptide aldehydes (and hydroxamates) using the same linker.[104] RA with methoxyamine hydrochloride in the presence of $NaBH_3CN$ provided a resin-bound methoxylamine. Acylation with Fmoc-amino acids, followed by solid-phase chain elongation, gave BAL-anchored peptide Weinreb amides. These could now be cleaved either acidolytic to provide peptide hydroxamates or by reduction with $LiAlH_4$ to yield the corresponding peptide aldehydes (hence, the latter may not be a BAL strategy in the strict sense, since the linkage is not through a backbone amide in the cleaved product). Treatment with TFA−iPr_3SiH−H_2O (95:2.5:2.5) resulted in crude yields of 68−83% and purities > 85%, whereas cleavage with $LiAlH_4$ in THF at 0 °C gave yields of 16−53% and purities in the range of 30−40%. A pair of diastereomeric hexapeptide aldehydes was prepared to assess racemization. With an estimated HPLC detection limit of 5%, the undesired epimers were not detected.

Scheme 17. Synthesis of Peptide Thioesters by Masking the C-Terminal Gly as a TTO Ester[65]



3.3.2. Peptide Thioesters

Peptide thioesters are widely used for native chemical ligation of unprotected peptide fragments.[105] As the thioester moiety is labile to the nucleophilic piperidine used in the repetitive removal of Fmoc, peptide thioesters have previously mostly been synthesized by the less prevalent Boc/Bn methodology. There are, of course, exceptions, including use of Kenner's sulfonamide "safety-catch" linker[106] employed by the groups of Pessi, Kent, Ellman, Bertozzi, and others using Fmoc chemistry.[107] This linker is stable to repetitive exposure to piperidine, while activation with diazomethane or iodoacetonitrile provides an *N*-alkyl acylsulfonamide, which is susceptible to nucleophilic attack from a thiol of choice. However, this method is hampered by the need for harsh alkylation conditions. The methods available for Fmoc-based solid-phase peptide synthesis of peptide thioesters have been reviewed.[108]

In the first reported BAL approach to peptide thioesters, the C-terminal of a BAL-anchored peptide was allyl protected during chain elongation, deprotected on-resin, followed by coupling of an amino acid thioester and release of the peptide from the resin.[67] Although this approach was successful in many cases, its scope was somewhat limited by the need for special precautions when deprotecting the second amino acid to prevent DKP formation and the possible racemization when coupling the amino acid thioester.

In an alternative BAL approach developed by our group, the thioester functionality was masked as a trithioortho (TTO) ester during chain elongation (Scheme 17; see also T-BAL synthesis under section 2.3.3).[65] Here, the sp^2 carbonyl carbon of the thioester was masked as the sp^3 carbon of a TTO ester during peptide-chain assembly. This eliminated the risk of DKP formation, and the final treatment with TFA−H_2O converted the TTO ester to the thioester with simultaneous release from the BAL handle. The key TTO derivative was obtained by treatment of the corresponding ester with $Al(SEt)_3$, either directly on a BAL-anchored glycine ester or on the glycine ester in solution. In the latter strategy, the resulting glycine TTO ester, $HCl \cdot H_2NCH_2C(SEt)_3$, was subsequently anchored to resin-bound trialkoxybenzyl BAL

by RA. A model peptide thioester, H-Phe-Val-Lys-Glu-Tyr-Ala-Gly-SEt, was synthesized in >90% purity by both strategies. RA with presynthesized TTO ester, however, gave better isolated peptide yield (42%). This is a general approach to the synthesis of peptide thioesters with a C-terminal Gly residue; peptide thioesters used in native chemical ligation often incorporate a sterically undemanding C-terminal Gly residue.

3.3.3. Other C-Terminally Modified Peptides and Conjugates

Nowick and co-workers synthesized the peptide amide iPr-CO-Phe-Hao-Val-NHBu on the indole linker, starting with RA of butylamine.[109] The unnatural Hao amino acid building block is 5-amino-2-methoxybenzoic acid hydrazide (a β-strand mimic) with an oxalamide linker. The peptide was also prepared on PAL, starting with alkylation of the PAL benzylamine with butyl iodide, but this was not the preferred strategy.

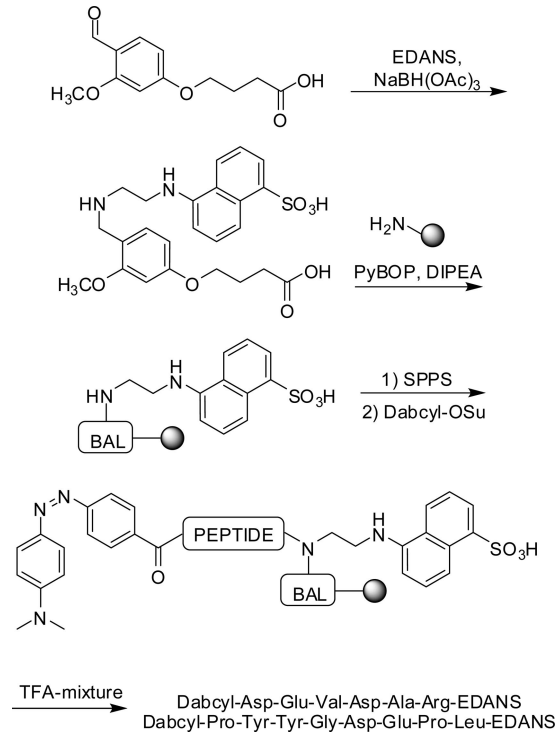
Hruby and co-workers reported the preparation of model amino acid hydrazide Bz-Gly-$NHNH_2$ on their 4-alkoxy-2-hydroxybenzaldehyde (AHB) linker (see discussion of this linker in section 2.1.4).[45] The linker, in the form of 4-(4-formyl-3-hydroxyphenoxy)butyric acid, was coupled to aminomethyl PS (AM−PS). Next, Boc-$NHNH_2$ was added to generate the hydrazone in toluene, followed by RA with $NaBH(OAc)_3$ in DMF. Coupling of Bz-Gly-OH was mediated with HBTU. The phenolic hydroxyl was then deacylated with piperidine−DMF (1:4) to make the linker more acid-labile. Still, HF was required to cleave the product, which was released in 64% yield.

Ellman and co-workers prepared C-terminally alkoxyamine-functionalized peptides with a trialkoxybenzyl BAL strategy, using $BocNHO(CH_2)_3NH_2$ for the RA.[110] Following assembly and cleavage from the solid hase, the peptides were immobilized on aldehyde-functionalized glass slide microarrays via oxime formation. Because the peptide sequences further contained the 7-amino-4-carbamoylmethyl coumarin probe, which becomes fluorescent after hydrolysis of the peptide anilide bond, the peptide array was used to determine protease substrate specificity.

Applying a 4-(4-formyl-3-methoxyphenoxy)butyrate BAL, an amphiphilic octapeptide was assembled with the β-hairpin sequence -Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly-, a C-terminal *n*-octadecylamine, and a N-terminal stearic acid.[111] Conformation of the peptide was studied and compared with identical sequences without the C-terminal modification or without both N- and C-terminal modifications. When the amphiphilic peptide was anchored in a liposome, the β-hairpin fold was stabilized.

As briefly mentioned in section 2.1.2, peptides with a C-terminal α-amido methylketone (−NHCO−$COCH_3$) have been prepared starting from commercially available NovaTag resin.[37] The linker on this resin is 4-(3-formyl-2-methoxyphenoxy)butyrate, derivatized by RA with monoprotected ethylenediamine. Following peptide assembly from the secondary amine, the N-terminal ethylenediamine was deprotected and acylated with pyruvic acid. Cleavage from the linker was performed with TFA−H_2O−PhOH (90:5:5) to release the 19-residues-long keto-peptide in 95% purity, 50% yield after HPLC. Cleavage with silane scavengers resulted in reduction of the ketone. The authors also concluded that application of base (such as piperidine) after introduction of the keto-functionality lead to lower product purity, presumably due to byproduct formation via a Claisen reaction. This was avoided by ending the peptide assembly with a Boc-amino acid and coupling the pyruvic acid with DCC/NHS. The keto-peptide product was subsequently ligated to an aminooxyacetyl-functionalized peptide sequence in aqueous buffer with pH 2.0.

Scheme 18. BAL Synthesis of Peptides Labeled for FRET Studies[112]

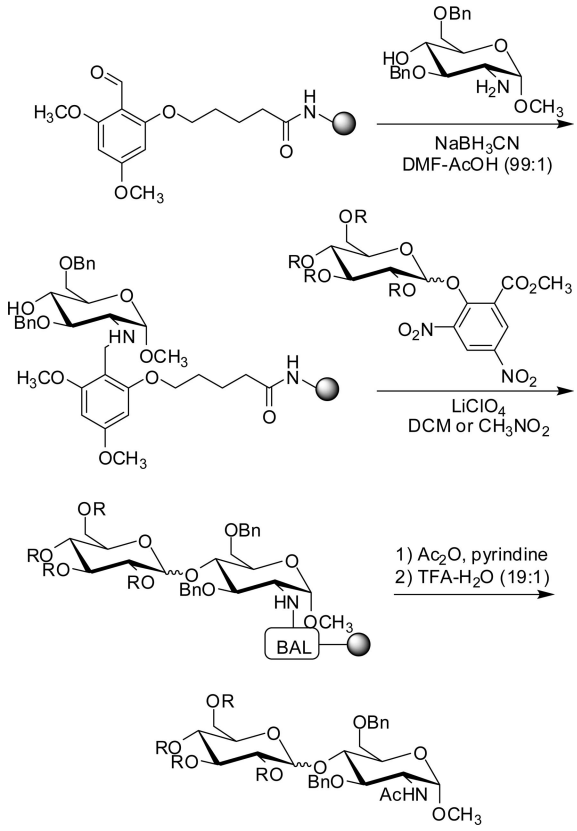


Beythien and White used a BAL strategy to prepare peptides C-terminally labeled with the EDANS fluorophore.[112] The EDANS group was coupled to 4-(3-formyl-2-methoxyphenoxy)butanoic acid (FMPB) by RA in solution with $NaBH(AcO)_3$ and $PhCH_2N(Me)_3OH$. The linker was then coupled to AM−PS resin, and peptide elongation proceeded by standard Fmoc methodology. The assembly was terminated by coupling the Dabcyl quencher to the N-terminus, providing peptides to be used for fluorescence resonance energy transfer (FRET) studies. Two sequences were obtained in good yields and excellent purities after cleavage with TFA-cocktails (Scheme 18). Barany's 2005 synthesis of C-terminal peptide hydroxamates by RA with methoxylamine has been described above.[104]

4. Applications in Oligosaccharide and Glycopeptide Synthesis

A safety-catch strategy for anchoring of carbohydrates in solid-phase oligosaccharide synthesis using trialkoxybenzyl *o*-BAL has been developed (Scheme 19).[113] Chemoselective anchoring of unprotected D-glucosamine, methyl 2-amino-2-deoxy-α-D-glucopyranoside, and partially *O*-protected glucosaminides through the 2-amino group to *o*-PALdehyde−PS resin proceeded smoothly by $NaBH_3CN$-mediated RA in AcOH−DMF (1:49). There were no indications of unintended formation of an *N*,*O*-acetal. As *O*-glycosylations often occur in the presence of strong Lewis acids, e.g., TMSOTf or BF_3−OEt_2, an acid-stable linkage was important during this reaction. The safety-catch strategy utilized that the linkage between BAL and the amine was stable to Lewis

Scheme 19. Solid-Phase Oligosaccharide Synthesis with Safety-Catch BAL Anchoring and DISAL Glycosyl Donors;[113] R = Bn, Bz



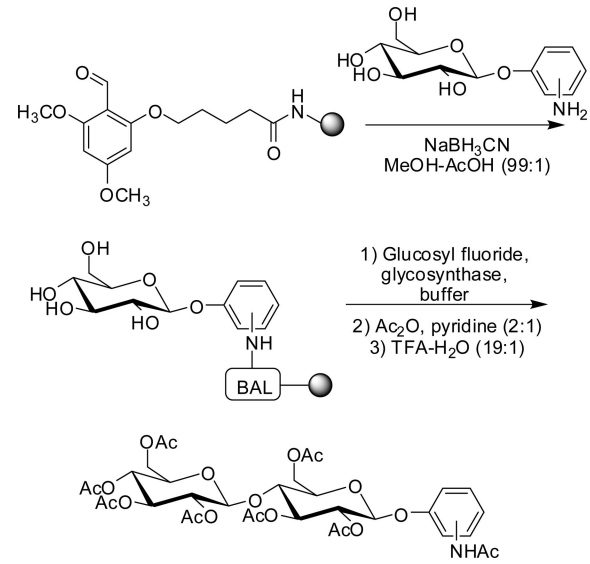
acids (and conc. TFA); however, upon activation by *N*-acylation, the amide could be released by treatment with TFA−H_2O (19:1). Solid support bound glucosamine derivatives carrying one free hydroxyl were glycosylated using either trichloroacetimidate (Tcai) or methyl 3,5-dinitrosalicylate (DISAL) glucosyl donors. Tcai donors required at least 2 equiv of TMSOTf as promoter, possibly due to the presence of the free amine. In contrast, glycosylation with DISAL glucosyl donors, carrying either benzyl or benzoyl protecing groups, proceeded in the presence of an excess of the very mild reagent $LiClO_4$. Following the *O*-glycosylation, the secondary 2-amino group was *N*-acetylated, and the disaccharides were released with TFA−H_2O (19:1).

A BAL handle has also been used for anchoring through the aglycon in enzymatic solid-phase oligosaccharide synthesis. Tolborg et al. attached aminophenyl glucosides by the aniline to a trialkoxybenzyl BAL on a highly swellable PEGA support.[114] They used engineered glucosidases (51 kDa mutants of *Agrobacterium sp.* β-glucosidase, Abg E358S and E358G), catalyzing the transfer of glucosyl fluorides to a 4-OH on glucosides forming a β-glycosidic linkage. It was demonstrated that these glycosynthases catalyzed glycosylations of resin-bound acceptor glycosides, with the highest yields for the 4-aminophenyl glycoside (Scheme 20). Furthermore, it was shown that BAL-anchored glycopeptides can act as acceptors after assembly on-resin, thus demonstrating a synthetically advantageous tolerance of acceptor structure.

5. Applications in Small-Molecule Synthesis

Solid-phase methodology has been applied extremely widely, extending it beyond the synthesis of oligomers like

Scheme 20. Solid-Phase Enzymatic Oligosaccharide Synthesis via Aglycon BAL Anchoring[114]



peptides, oligonucleotides, and oligosaccharides, to also become a general tool for the synthesis of small nonoligomeric molecules. BAL anchoring can readily be adapted to nitrogen-containing organic molecules other than amino acids, allowing for significant applications to solid-phase organic synthesis and combinatorial chemistry. For example, Krchňák and Holladay have reviewed solid-phase heterocyclic chemistry, which includes some BAL examples.[115] Also on a related subject, *N*-alkylation reactions and indirect formation of amino functionalities in solid-phase synthesis have been reviewed,[116] while another recent review focused on strategies for solid-phase synthesis of hydroxamic acid derivatives.[117]

5.1. Amines, Amides, and Others

5.1.1. Amines, Amides, and Hydrazides

In most BAL strategies, the BAL-anchored secondary amine is acylated after its anchoring to the linker, which also provides a better leaving group, amide rather than amine, for the final cleavage step. In effect, this ensures high purity of the released amide, even with incomplete acylation. However, the release of secondary amines from a trialkoxybenzyl BAL with TFA has been reported by Albericio and co-workers,[118] who anchored primary amines by standard RA and then converted them to secondary amines (or tertiary amines, if counting the BAL benzyl) by either a second RA with another aldehyde or a Petasis multicomponent reaction with glyoxal and a boronic acid. Different cleavage conditions were investigated: TFA−DCM (95:5, 16 h) gave 70−100% cleavage yields and 90−98% HPLC purity. In view of the very significant interest in the synthesis of secondary amines for medicinal chemistry, it is noteworthy that this has not been used more. The mechanism for acidolytic release of secondary amines is different from that for the release of amides due to the absence of an amide carbonyl. Secondary amines have also been prepared using a T-BAL handle, however, in lower yields (see under section 2.3.3).[61]

In contrast, it has been more widely utilized that aromatic amines (anilines) can be cleaved from BAL handles. This was, for example, used to monitor reactions in a study aimed at preparing a small library of mimetics of the cyclic

depsipeptide hapalosin, using 3-amino-4-hydroxy-5-nitrobenzoic acid as scaffold and without protecting groups.[119] With the 5-(4-formyl-3,5-dimethoxyphenoxy)pentanoic acid BAL on PS, RA anchored the anilino functionality. The carboxylic acid was activated with PyBOP and amidated with 2-aminopropane or 2-methyl-1-aminopropane. A PyBOP-phenol adduct was observed, but treatment with NaOMe removed this *O*-modification. Next, the BAL secondary amine was acylated with hexanoyl or heptanoyl chloride, and the nitro group was reduced with $SnCl_2 \cdot 2H_2O$. The newly formed anilino functionality was finally derivatized by acylation with benzoyl or phenylacetyl chloride (again followed by NaOMe treatment to remove *O*-modification from the phenol hydroxy), or by RA with benzaldehyde. The completed structures (**3**) were cleaved from the linker with TFA−H_2O (19:1) and purified by flash chromatography with yields in the range 10−40%.

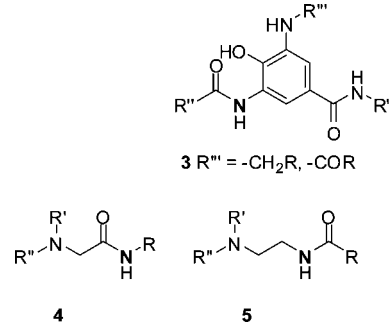
Albericio, Barany, and co-workers have outlined the potential of BAL-anchored scaffolds with a few examples covering DKPs, hydrazines/hydrazides, and alkylenediamines/amides.[78] For the hydrazines, it was suggested to perform RA with monoprotected hydrazine (e.g., Ddz-protection). Subsequent acylation would then be selective for the α-nitrogen, whereas the *β*-nitrogen could be exposed after deprotection for a second acylation reaction. For the alkylenediamines, the Trt- or Fmoc-monoprotected reagents were envisioned for the RA, giving the same possibilities for selective introduction of diversity elements. Building on this approach, Barany and co-workers in 2003 published the synthesis of 60 lidocaine (**4**) and procainamide (**5**) analogues from 5-(4-formyl-3,5-dimethoxyphenoxy)pentanoic acid on AM PEG−PS resin.[120] Both sets of analogues could be prepared using RAs, acylations, and displacement of halide with an amine. The structures were cleaved from the linker with TFA−H_2O (19:1) in crude purities >80% and isolated in 40−88% yields.

In a communication, the use of unprotected diamines was described, now in acylation reactions with BAL-anchored benzoic acid derivatives.[121] The authors concluded that the nature of the resin and the length of the linker arm on the BAL handle play only minor roles in acylation selectivity (monoacylation versus diacylation of the diamine via cross-linking). Monoacylation could be selectively achieved for a range of unprotected symmetrical diamines by employing a high concentration of the diamine (1 M, 20 equiv).

Researchers from Novartis have applied BAL for the synthesis of malondiamides.[122] The optimized procedure was found to be RA with a primary amine, followed by acylation with dialkyl malonyldichlorides. This generated an intermediate (mono)acid chloride, which could be reacted with a range of primary and secondary amines to form the target compounds (**6**). The authors concluded that malonylpeptidomimetics offer advantages in comparison with natural peptides. Reacting a dimethyl malonyldichloride activated resin with unprotected phenylalanine yielded peptidomimetic in good purity (>85%) after acidolytic cleavage from the resin.

A rhodamine- and maleimide-derivatized alkylamine was assembled by anchoring tetradecylamine by RA to 4-(4-formyl-3-methoxyphenoxy)butyryl aminomethyl (FMPB AM) resin.[123] Next, acylation with Fmoc-Lys(Mtt)-OH was followed by selective deprotection and functionalization of the two Lys amines with the fluorophore and ε-maleimidocapric acid. This building block was later joined with a Cys-containing structure, assembled on Rink resin, in a convergent synthesis of a membrane permeable fluorophore-appended protein tyrosinase phosphatase 1B (PTP1B) inhibitor (see Chart 4).

Chart 4. Selected amide structures; the bold nitrogen indicates the point of attachment to BAL.



5.1.2. Sulfonamides, Carbamates, Hydroxamic Acids, and Ureas

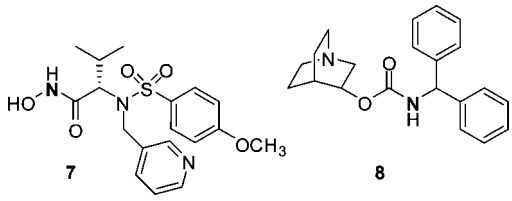
Functionalization of BAL-anchored secondary amines can be performed not only by acylation to provide secondary amides but also by reaction with other electrophiles, thereby creating new backbone structures. An early example was reported by Fivush and Wilson,[8] as previously discussed under section 2.1.2. After anchoring a primary amine to the AMEBA resin by RA, the resulting secondary amine was treated with *p*-toluenesulfonyl chloride to produce a sulfonamide. The structure was cleaved from the support with TFA−DCM (5:95) in 66% yield and 95% purity. The authors report that the high purity was unexpected and prompted them to further studies, which revealed that the unmodified amine could not be cleaved from the linker even with TFA−H_2O (95:5). However derivatized as a sulfonamide, amide, urea, or carbamate, the products were easily released.

Likewise, Swayze reported the derivatization of ArgoGel through a Mitsunobu reaction with 4-hydroxybenzaldehyde and 4-hydroxy-2-methoxybenzaldehyde.[9] Subsequent RA with cyclohexylamine was performed as a two-step procedure with preformation of the imine before reduction with $BH_3 \cdot$pyridine, catalyzed by AcOH (see also under section 2.1.2). The BAL-anchored secondary amine was then reacted with tolyl isocyanate, tosyl chloride, and three different activated carboxylic acids, to provide the urea, sulfonamide, and amide products, respectively. Cleavage was possible from both linkers with TFA−Et_3SiH (97.5:2.5), but was faster for the urea and sulfonamide compared to the amide structure (which required up to 18 h to be released from the linker without methoxy-substituents).

In an early application of a BAL handle to the synthesis of hydroxamic acids,[124] 4-(4-formyl-3,5-dimethoxyphenoxy)-butyric acid was reacted with *O*-protected hydroxylamines in solution to form the oximes, which were reduced in situ with $NaBH_3CN$ in AcOH for 18 h. Both allyl and tetrahydropyran (THP) were evaluated as *O*-protecting group. After attachment to solid phase, the BAL secondary amine was acylated. With the THP group, deprotection and cleavage from the linker occurred simultaneously by treatment with TFA. The methodology was used to synthesize a known matrix metalloproteinase (MMP) inhibitor, CGS 27023A (**7**, Chart 5).

The synthesis of *N*-substituted carbamates has been studied by Albericio and co-workers.[31] After RA of resin-bound 4-(4-

Chart 5. Examples of sulfonamide and carbamate structures.



formyl-3,5-dimethoxyphenoxy)butyric acid, carbonylation was obtained with an alkyl succinimidyl carbonate, prepared in situ from a large excess of the alcohol and *N*,*N*′-disuccinimidyl carbonate (DSC) and catalyzed by DMAP. After RA with benzhydrylamine, a carbonylation reaction with quinuclidinol had to be heated to 70 °C for the reaction to take place. Final cleavage with TFA−$CHCl_3$−H_2O (50:50:1) was reported to be cleaner than the standard TFA−H_2O (95:5). Quinuclidinol carbamates (**8**, Chart 5) were obtained in 40−75% yield and 85−99% purity.

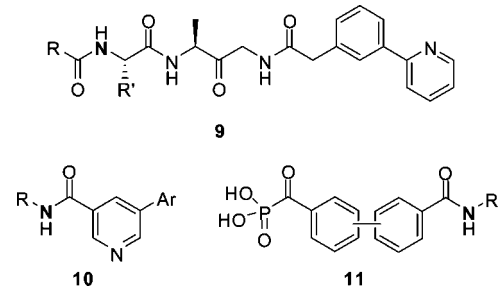
Another research group in Barcelona investigated the synthesis of *N*,*N*′-disubstituted sulfonamides, R′NH-SO_2−NHR.[125] After RA with an aliphatic or aromatic amine (H_2N−R), the BAL secondary amine was derivatized with the −SO_2−NHBoc. The *N*-monosubstituted products could then be cleaved from the linker. Alternatively the Boc-protected nitrogen was alkylated with a range of alcohols (R′−OH) under Mitsunobu conditions. Concomitant cleavage and Boc-removal provided *N*,*N*′-disubstituted sulfonamides (see Chart 5).

5.1.3. Other Functionalities

Veber and co-workers reported the synthesis of a small library of 1,3-bis(acylamine)-2-butanones for evaluation of cysteine protease inhibitors.[126] Applying the 3,5-dimethoxybenzyl BAL, amino acid methyl esters were anchored by RA, and the BAL secondary amine was acylated with a carboxylic acid. Next, the methyl ester was hydrolyzed with $KOSiMe_3$, and the free carboxylic acid was coupled to 1-azido-3-amino-2,2-dimethoxybutane, $H_2NCH(CH_3)C(OCH_3)_2CH_2N_3$. The azide was then reduced with $SnCl_2$/PhSH, and the resulting primary amine was acylated with 3-(2-pyridyl)phenylacetic acid. Final cleavage from the linker occurred with concomitant hydrolysis of the ketal to form the desired structures (**9**, Chart 6). Using three different amino acid esters and six different carboxylic acids, an 18-membered library was prepared. The authors report some epimerization of the butanone (∼20%), presumably occurring during hydrolysis of the ketal.

Solid-phase Suzuki coupling reactions have been explored using BAL chemistry. Albericio and co-workers synthesized a 180-member library of 5-substituted nicotinic acid derivatives (**10**, Chart 6) with this approach.[127] RA with a primary amine was followed by acylation with 5-bromonicotinic acid and Suzuki coupling using a range of boronic acids. Cleavage with TFA−DCM (1:1, 15 min) produced the desired products in 75−85% yield and 71−96% purity. Furthermore, in a 2006 publication, the solid-phase synthesis of biaryl α-ketophosphonic acids (**11**, Chart 6) was reported.[128] The BAL handle was derivatized with one of six different primary amines, followed by acylation with 3- or 4-carboxylphenylboronic acid (see Chart 6). In a microwave-mediated aqueous Suzuki coupling, the polymer-bound boronic acids were reacted with one of three α-ketophosphonic acids. After

Chart 6. Selected small molecules prepared by a BAL strategy.



acidolytic cleavage from the resin with TFA−DCM (1:1, 20 min), the products were purified by HPLC to give 20−30% isolated yields.

5.2. Heterocycles

BAL strategies are obvious for solid-phase assembly of a large variety of *N*-heterocycles.[115,129] Indeed a number of diverse applications have been reported, including examples of BAL-anchoring through in-ring nitrogens with on-resin cyclization. In the following sections, the literature reports are briefly described, starting with heterocycles containing a single nitrogen (section 5.2.1) and followed by structures with two or more nitrogens (sections 5.2.2−5.2.11); finally reports on cyclic structures containing nitrogen in combination with other heteroatoms are presented (section 5.2.5).

5.2.1. Piperidines

Albericio and co-workers have used 5-(4-formyl-3,5-dimethoxyphenoxy)pentanoic acid as a handle for the synthesis of substituted 4-aminopiperidines.[130] 4-Amino-1-alloc-piperidine was prepared in solution starting from piperidone and was anchored to the linker by RA. The BAL secondary amine was then acylated, the Alloc group was removed, and the piperidine nitrogen was derivatized, before acidolytic cleavage from the resin. Conditions for Alloc removal were systematically investigated, and superior (quantitative) results were obtained with $Me_2NH\cdot BH_3$ as scavenger for the allyl cations in deprotections with Pd-$[PPh_3]_4$.

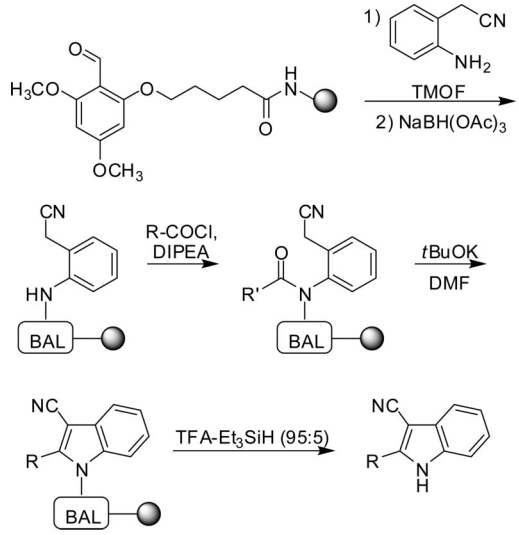
5.2.2. Indoles

A modified Madelung solid-phase synthesis of 2,3-disubstituted indoles was demonstrated (Scheme 21).[131] Using commercially available BAL resin, 2-aminophenylacetonitrile was anchored by RA. The BAL amine was then acylated with a carboxylic acid chloride, while the critical cyclization was best performed with *t*-BuOK in DMF over 2 h. Cleavage with TFA−Et_3SiH (95:5) over 30 min gave the indole in 88% yield and 95% purity. A range of different anilines and carboxylic acid chlorides (both aromatic and aliphatic) were evaluated with good results. It was also demonstrated that cyclization can be accomplished with an ester or amide functionality in place of the nitrile.

5.2.3. Piperazines

Herpin et al. published the preparation of a 10 000-member library on the piperazine−2-carboxylic acid scaffold.[132] By applying a 4-formyl-3,5-dimethoxyphenoxy linker, preformed *N*-Fmoc, *N*′-Alloc protected piperazine-2-carboxylic acid was used to acylate the BAL secondary amine formed by the

Scheme 21. BAL Synthesis of 2,3-Substituted Indoles[131]

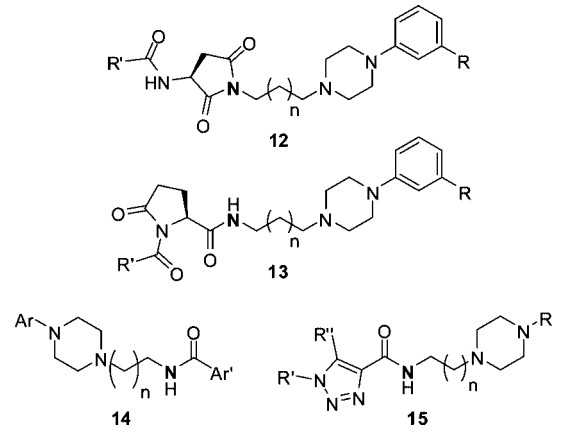


initial RA with primary amines. The protecting groups were removed sequentially, followed by reaction with sulfonyl chlorides, isocyanates, chloroformates, and carboxylic acids to afford sulfonamides, ureas, carbamates, and amides, respectively.

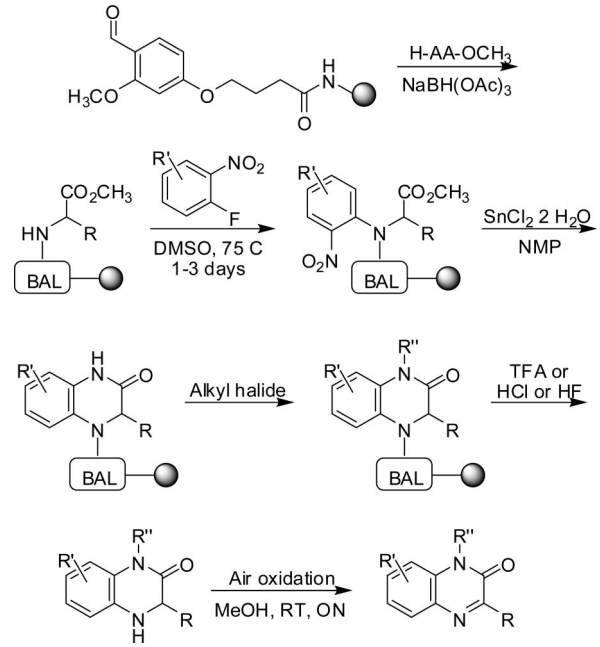
A 72-membered *N*-aryl piperazine library was synthesized by Zajdel and co-workers on commercially available BAL (4-formyl-3,5-dimethoxyphenoxyacetic acid) Synphase Lanterns.[133] The starting material for the synthesis was substituted *N*-aryl-*N*′-aminoalkyl piperazines. After RA, Fmoc−Asp(*t*-Bu)−OH or Fmoc−Glu(*t*-Bu)−OH was used to acylate the BAL amine. Subsequent to Fmoc-deprotection, the amino group was derivatized with a carboxylic acid. Then, by applying TFA−$CHCl_3$−$SOCl_2$ (50:50:1.5) at 40 °C over 10 h, the structures could be finalized in a one-pot cleavage/cyclization proces, in which the Asp derivatives formed succinimides (**12**) and the Glu derivatives cyclized to pyroglutamyls (**13**). A year later (2005), the same authors reported the synthesis of a similar 64-membered library now with a proline ring system, again using BAL Synphase Lanterns.[134] Hence, the syntheses began with RA with aminoalkyl piperazine or aminoalkyltetrahydropyridines. Next, Fmoc−Pro−OH (L or D) was coupled to the BAL amine, followed by Fmoc-removal and reaction with a range of acyl or sulfonyl chlorides to give carboxamide and sulfonamide products, respectively.

Gmeiner and co-workers have used their formyl indolyl methyl triazole (FIMT)[59] and formyl aryl oxymethyl triazole (FAMT) resins, prepared by Huisgen 1,3-dipolar cycloaddition, in the synthesis of aryl piperazine carboxamides.[135] Aminoalkyl piperazines were anchored to the BAL-type handles by RA. In one strategy (on FIMT linkers), this was followed by acylation with aromatic carboxylic acids, while the piperazine functionality was utilized for Pd-catalyzed *N*-arylations (**14**). In the other strategy (on FAMT linkers), the secondary amine was acylated with different activated alkynoic acids, used for [3 + 2]-cycloadditions employing benzyl azides as 1,3-dipolar agents, to form 1,2,3-triazole carboxamides (**15**). Both methodologies were utilized to synthesize libraries (42 and 60 members, respectively), before cleavage with 2% TFA in DCM afforded the desired compounds in acceptable yields and good purities (see Chart 7).

Chart 7. Selected piperazine structures prepared by BAL chemistry.



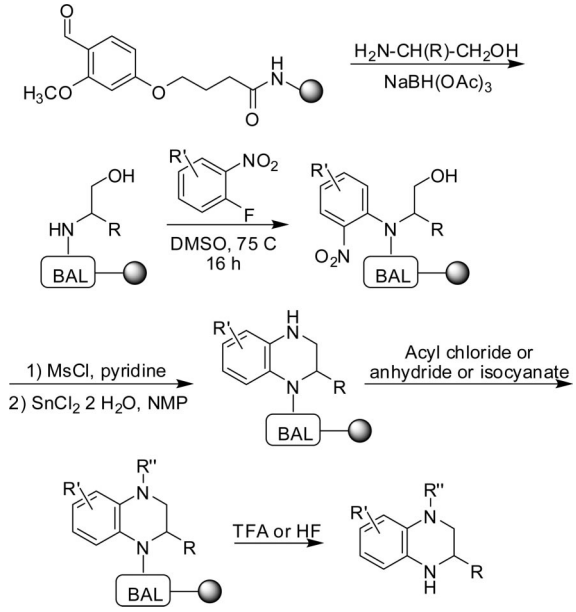
Scheme 22. BAL Synthesis of Quinoxalinones[136]



5.2.4. Quinoxalines

Krchňák and co-workers have described the synthesis of quinoxalinones[136] and tetrahydroquinoxalines[137] in two communications. The starting point for both syntheses was 4-(4-formyl-3-methoxyphenoxy)butyryl AM resin. Assembly of the quinoxalinones (Scheme 22) then followed a route consisting of RA with an amino acid ester, nucleophilic fluorine displacement by reaction of *o*-fluoronitrobenzene with the BAL secondary amine, and reduction of the nitro group with $SnCl_2$ to the amine, which resulted in spontaneous cyclization to the dihydroquinoxalinone. The amide functionality could then be alkylated before the structures were cleaved from the linker with TFA (or HCl or HF), which caused air oxidation to the quinoxalinone. It was reported that the nucleophilic aromatic substitution (in DMSO at 75−90 °C) resulted in <10% yield when tested on amino acids with bulky side chains or *o*-fluoronitrobenzenes with no additional electron-withdrawing substituents. The tetrahydroquinoxalines were assembled by a similar strategy (Scheme 23), starting with RA with a β-amino alcohol, and then reacting the BAL nitrogen with *o*-fluoronitrobenzenes. In order to close the six-membered ring, the alcohol was converted to a mesylate before reduction of the nitro group.

Scheme 23. BAL Synthesis of Tetrahydroquinoxalines[137]



After spontaneous cyclization, the aniline nitrogen could be derivatized by acylation, alkylation, or urea formation. The finished tetrahydroquinoxalines were cleaved with TFA or HF in 84−95% yield.

5.2.5. Benzodiazepines

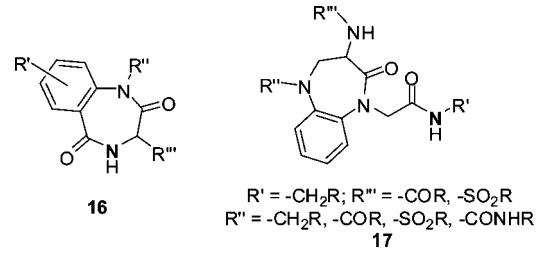
In 1995, Ellman and co-workers reported the solid-phase synthesis of 1,4-benzodiazepine-2,5-diones (**16**) on 4-hydroxy-2,6-dimethoxybenzaldehyde bound to chloromethyl PS (Merrifield resin).[33] The synthesis was initiated by RA with an amino acid ester, followed by acylation with a 2-aminobenzoic acid. Base-catalyzed lactamization could now be performed by lithium salts of acetanilide or *p*-methoxyacetanilide. This resulted in the anilide anion of the lactam, which was *N*-alkylated in the same reaction step. For some structures, further diversity was added through a Suzuki cross-coupling reaction. The benzodiazepines were cleaved with TFA−Me_2S−H_2O (90:5:5) in very acceptable yields. The strategy was later used to construct a 2 508-member library in microtiterplate format.

A 10 530-member 1,5-benzodiazepine-2-one (**17**) library was prepared on a 4-formyl-3,5-dimethoxyphenoxy linker.[138] Initially a primary amine was anchored by RA, followed by acylation with bromoacetic acid. Next, a 3-amino-1,5-benzodiazepine-2-one scaffold prepared in solution (with the 3-amine protected as a phthalimide) was added, resulting in alkylation of the N1. The N5 could then be derivatized through reaction with benzylic halides, acyl halides, sulfonyl chlorides, or isocyanates, followed by deprotection and derivatization of the 3-amine by acylation or sulfonylation. The library was produced as an 18 × 13 × 45 array using the Irori system. The cleaved and analyzed products in general showed >75% purity (see Chart 8).

5.2.6. Purines

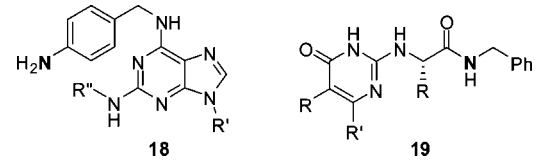
In an early report, a BAL-anchored purine scaffold was derivatized to prepare a small set of 2,9-substituted purines (**18**).[139] The scaffold, 2-fluoro-6-(4-aminobenzylamine)purine, was anchored by RA to 5-(4-formyl-3,5-dimethoxyphenoxy)pentanoic acid derivatized AM resin. The first combinatorial step was then selective alkylation of the purine

Chart 8. Benzodiazepine structures.



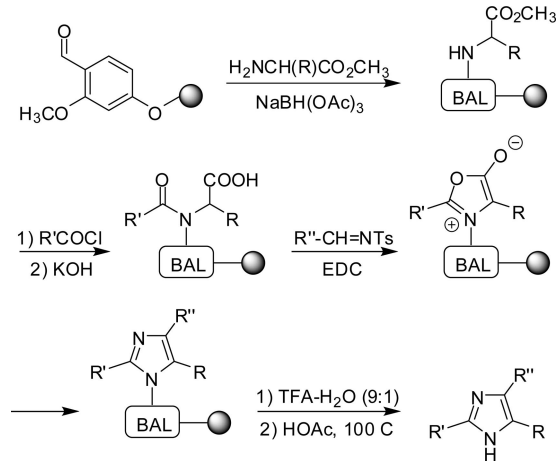
16 17

Chart 9. Other heterocyclic structures.



18 19

Scheme 24. BAL Synthesis of Imidazoles by Münchnone (3 + 2)-Cycloaddition[141]



N9-position under Mitsunobu conditions using a range of primary and secondary aliphatic alcohols (R′−OH). The second combinatorial step consisted of F-substitution in the C2-position with amines (R″−NH_2). The final products were cleaved with TFA−H_2O−Me_2S (95:5:5) in 51−85% HPLC yield.

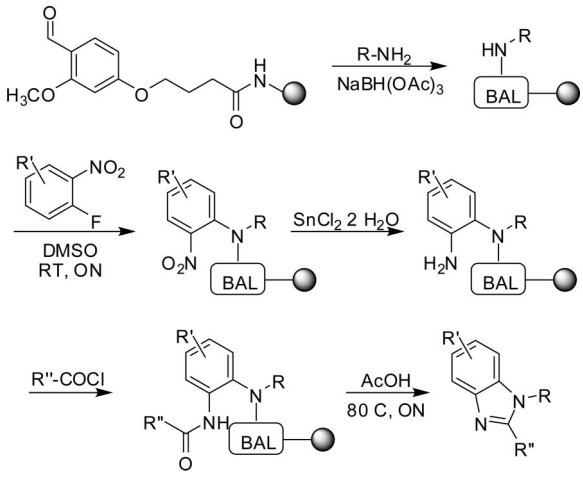
5.2.7. Pyrimidinones

Zapf and Goodman used 2,6-dimethoxy-4-hydroxybenzaldehyde coupled to bromoalkyl-derivatized Tentagel resin to prepare 2-amino-4-pyrimidinones from guanidines (**19**).[140] RA with benzylamine was followed by acylation with Fmoc-protected amino acids. After deprotection, the amine was guanidinylated, and subsequent reaction with β-keto esters yielded the desired heterocyclic structures in good purities (see Chart 9).

5.2.8. Imidazoles

Researchers from Merck reported in 1998 the solid-supported synthesis of a small library of triarylimidazoles (Scheme 24).[141] Using ArgoGel−MB−CHO (4-formyl-3-methoxyphenoxy) resin, an amino acid methyl ester was anchored by RA. The amine was then acylated with a carboxylic acid chloride, and the methyl ester was saponificated. The key step was now a münchnone (3 + 2)-cycloaddition, in which the resin-bound acid was treated with a carbodiimide (EDC) and a tosylimine to give the imidazole

Scheme 25. BAL Synthesis of Benzimidazoles[142]



over 24−48 h. The authors note that the imidazole was not cleaved from the linker with TFA−H_2O (9:1), which could be used as a purification step, because unreacted starting material and nonimidazole byproducts were retained under these conditions. The products could, however, be cleanly liberated from the solid phase with glacial AcOH (100 °C, 2 h). A 12-member library (2 × 3 × 2) was produced, with average yields of 73% and purities of 96%.

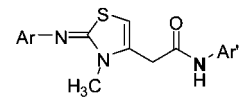
5.2.9. Benzimidazoles

Krchňák and co-workers also reported synthesis of benzimidazoles[142,143] on 4-(4-formyl-3-methoxyphenoxy)butyryl resin, using a similar strategy as for the assembly of quinoxalinones.[136] Hence, after RA, the BAL amine was reacted with *o*-fluoronitrobenzene, and the resulting *o*-nitroaniline was reduced with $SnCl_2$. Acylation with a carboxylic acid chloride was followed by treatment with AcOH at 80 °C overnight, which resulted in cleavage from the linker and cyclization to the benzimidazole (Scheme 25). Alternatively, acylation with a 4-chlorophenyl isothiocyanate formed the thiourea, which, after acidic release from the linker, could be cyclized in solution with DIPCDI to yield a 2-arylaminobenzimidazole.

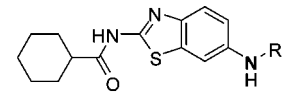
5.2.10. Thiazolines

A monomethoxybenzyl linker was applied in the synthesis of fungitoxic 2-imino-1,3-thiazolines (**20**).[144] Following RA with anilines and acylation with chloroacetoacetyl chloride, condensation with thiourea derivatives gave the desired structures. A 20-member focused library was created, and the compounds were obtained in 38−95% yield after cleavage with TFA−DCM (1:1, 30 min). Short cleavage time was found critical to obtain good purity.

Chart 10. *N,S*-Heterocyclic structures.



20



R = -CO-R', -CO-OR', -CO-NHR', -CS-NHR', -SO_2-R'

21

5.2.11. Benzothiazoles

Another combinatorial example was the synthesis of benzothiazole derivatives (**21**) for evaluation as antitumor agents.[145] Here, an anilinobenzothiazole was coupled to FMPB AM PS resin by standard RA. The secondary amine was then acylated with 200 different reagents to give a range of amide, carbamate, urea, thiourea, and sulfonamide derivatives (see Chart 10).

6. Conclusions, General Principles, And Experimental Guidelines

The general backbone amide linker (BAL) strategy has allowed the particularly straightforward synthesis of linear C-terminal modified peptides, cyclic peptides, oligosaccharides, and numerous nitrogen-containing small organic molecules. Almost all BAL handles release the substrate under acidic conditions, where the introduction of electron-rich core structures and fine-tuning with electron-donating or -withdrawing groups modulate the acid-lability of the linker, e.g., toward releasing the substrates under very mild conditions. Outstanding features of the BAL strategy are the high-yielding steps, including easy attachment of amines by RA, stability of the BAL anchor to numerous chemical manipulations, and the often relatively mild conditions for final release of products. The synthesis of BAL has been performed by direct on-resin functionalization; however, the preferred strategy is conjugation of the complete linker molecule to the resin by straightforward amide bond formation. BAL appears to be compatible with all supports generally used for solid-phase synthesis. The focus of work

Table 1. Lability Chart: Conditions Reported for Acidolytic Release of Substrates from BAL-Type Handles; Note that, For Some Applications, The Reported Cleavage Conditions May Not Have Been Optimized

leaving group → / linker ↓	sulfonamide	urea	carbamate	amide	aniline	amine
monoalkoxybenzyl BAL				HF−*p*-cresol 10:1[82] or TFMSA−TFA 1:9, 2 h[25]		
monoalkoxyalkylbenzyl BAL				TFA, reflux, 3 h[42]		
dialkoxybenzyl BAL	TFA−DCM 5:95, 5 min[8]	TFA−TES 97.5:2.5, 15 min[9]	TFA−DCM 1:19[8]	TFA−DCM 3:7, 10 min[10]		
trialkoxybenzyl BAL	TFA−$CHCl_3$−H_2O 50:50:1, 1 h[125]		TFA−$CHCl_3$−H_2O 50:50:1, 1 h[130]	TFA−DCM 5:95−1:99, 1−2 h[36]	TFA−H_2O 19:1, 2 h	TFA−DCM 95:5, 16 h[118]
thiophene BAL[61]				TFA−DCM 1:99, 2 h		TFA−DCM 1:1, 2 h, 60 °C
indole BAL[57]	TFA−DCM 1:99, 3 min	TFA−DCM 1:99, <2 min	TFA−DCM 1:99, 1 min	TFA−DCM 1:99, 8 h		

done to date in the authors' laboratories has been on new acid-labile BAL handles, new chemistry using BAL handles, e.g., for the synthesis of peptide thioesters and aldehydes, and the application of BAL handles for the synthesis of peptides and oligosaccharides.

In the following, we have selected a few suggestions:

(1) The most common and popular BAL handles for the Fmoc-based synthesis of peptides are trialkoxybenzyl, dialkoxybenzyl, and indole linkers. While the dialkoxybenzyl linker might be less expensive than the corresponding trialkoxybenzyl, the latter is likely to offer better RA and acidolytic release of final products. All other things being equal, the aldehyde linker should be attached to the resin via a spacer (at least two carbons before the carbonyl) rather than by direct alkylation of a benzaldehyde derivative with chloromethyl resin. The monoalkoxybenzyl linker typically requires HF or TFMSA for release of peptides and, thus, is compatible with Boc chemistry. For a linker with a slightly higher acid-lability than trialkoxybenzyl, we recommend T-BAL.

(2) If possible, the use of 10 equiv each of amine and $NaBH_3CN$ is recommended. In some cases, as little as 1−2 equiv of amine will give efficient incorporation. However, high concentrations of amine will be more important than the number of equivalents. $NaBH(OAc)_3$ can be used instead of $NaBH_3CN$, and a range of other reducing agents have also been used. Often, reactions are performed at ambient temperatures, but microwave heating to 60 °C in a closed container can shorten reaction times and/or improve yields significantly.

(3) MeOH is well-suited as a solvent for RAs carried out either in solution or on-resin; for on-resin RAs, this typically requires a hydrophilic resin such as TentaGel, ArgoGel, PEG-PS, or PEGA. DMF and related solvents are also excellent for on-resin RAs and can be used when the support is PS. However, DMF is incompatible with the corresponding reactions *in solution*, as it promotes dialkylation of the amine. Mixtures of MeOH with other solvents have also been used successfully.

(4) When starting with an amine salt, often no additional acid is required for successful RA. When starting with a free amine, 1% HOAc is suggested for efficient incorporation.

(5) When incorporating a chiral amino acid derivative, a separate imine-forming step should be avoided.

(6) Acylation of the resin-bound secondary amine can be challenging. The preferred solvent is DCM or DCM−DMF (9:1), while neat DMF as solvent in some cases gave lower yields. Symmetrical anhydrides are often efficient and convenient acylating agents providing high yields; however, other coupling reagents, including HATU, have also been employed.

(7) The acidolytic release depends on the nature of the final product. In general, the order of decreasing acid-lability is as follows: sulfonamide > carbamate ≈ urea > secondary amide > primary amide > amine.

(8) BAL handles can be operated in a safety-catch mode, either by using the higher acid-stability of the BAL-anchored amine or by introduction of a temporary electron-withdrawing group on the linker. Steric effects are likely to play a role in the acidolytic release due to ground-state destabilization by steric congestion, which, for example, plays a role in the higher acid-lability of secondary amides over primary amides (see Table 1).

7. Acknowledgments

We are grateful to Professors Fernando Albericio and George Barany and Drs. Michael F. Songster, Josef Vagner, and Jordi Alsina, who all were coauthors on the original BAL publication, for inspiring discussions over the years. We also acknowledge our collaborator Professor Jørn B. Christensen for valuable contributions.

8. List of Abbreviations

AHB	4-alkoxy-2-hydroxybenzaldehyde
Alloc	allyloxycarbonyl
ALOBAL	allyloxyalkoxy BAL
AMEBA	acid-sensitive methoxybenzaldehyde
AM	aminomethyl
BAL	backbone amide linker
9-BBN	9-borabicyclo[3.3.1]nonane
Boc	*tert*-butyloxycarbonyl
BOP	benzotriazol-1-yl-*N*-oxy-tris(dimethylamino)phosphonium hexafluorophosphate
CMPI	2-chloro-1-methylpyridinium iodide
Dabcyl	*N*-(4-[4′-(dimethylamino)phenylazo]benzoyl
Dapa	2,3-diaminopropionic acid
DBU	1,8-diazabicyclo(5.4.0)undec-7-ene
DCM	dichloromethane
Ddz	α,α-dimethyl-3,5-dimethoxybenzyloxycarbonyl
DISAL	methyl 3,5-dinitrosalicylate
DIPCDI	*N*,*N*′-diisopropylcarbodiimide
DIPEA	*N*,*N*-diisopropylethylamine
DKP	diketopiperazine
DMA	1,1-dimethylallyl
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DSC	*N*,*N*′-disuccinimidyl carbonate
EDANS	5-(2-aminoethyl)amino-1-naphthalene-sulfonic acid
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
EDOT	3,4-ethylenedioxythiophene
FAMT	formyl aryl oxymethyl triazole
FIMT	formyl indolyl methyl triazole
Fmoc	9-fluorenylmethoxycarbonyl
FMPB	4-(4-formyl-3-methoxyphenoxy)butyryl
HATU	*N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide
HBTU	*N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide
HMBA	4-hydroxymethylbenzoic acid
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole
IRAA	internal reference amino acid
Ms	methylsulfonyl
Mtt	4-methyltrityl
NAL	naphthalene amide linker
NBS	*N*-bromosuccinimide
NHS	*N*-hydroxysuccinimide
NMP	1-methyl-2-pyrrolidinone
Nosyl	2-nitrobenzenesulfonyl
ON	overnight
PAL	peptide amide linker
PEG	polyethyleneglycol
PhiPr	phenylisopropyl
PS	polystyrene
PyAOP	7-azabenzotriazol-1-yl-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate
PyBOP	benzotriazole-1-yl-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate

PyBrOP	bromotris(pyrrolidino)phosphonium hexafluorophosphate
RA	reductive amination
RT	room temperature
SPPS	solid-phase peptide synthesis
T-BAL	thiophene BAL
TBAF	tributylammonium fluoride
TFA	trifluoroacetic acid
TFMSA	trifluoromethane sulfonic acid
THP	tetrahydropyran
TMOF	trimethyl-*ortho*-formate
TMSOTf	trimethylsilyloxytrifluoromethane sulfonate
Trt	trityl(triphenylmethyl)
TTO	trithio-*ortho*-(ester)
Z	benzyloxycarbonyl

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CR068206R