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New Stable Backbone Linker Resins for Solid-Phase Peptide Synthesis

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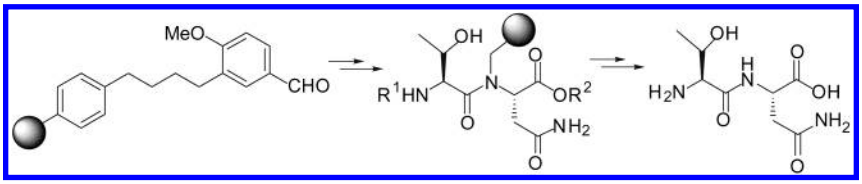
ABSTRACT



Two new 4-methoxybenzaldehyde backbone linker resins were developed for the solid-phase synthesis of peptides. The linkers are very stable during the cleavage of common protecting groups for amines (Fmoc, Boc) and carboxylic acids (Me, All, *t*Bu) in peptide synthesis. Cleavage from the resin with refluxing TFA is sufficiently mild for peptides containing polar and nonpolar amino acids.

In 1963, Merrifield reported the concept and initial demonstration of solid-phase peptide synthesis (SPPS).[1] SPPS has developed into a convenient and popular method for the synthesis of peptides and other small molecules. The key feature of solid-phase synthesis is the linker element, which is a molecule that keeps the intermediates bound to the support during solid-phase synthesis. Linkers should allow easy attachment of the starting material to the support, be stable under a broad variety of reaction conditions, and yet enable selective cleavage at the end of a synthesis without damage to the product. Many linkers have been developed for SPPS, among which the backbone linker strategy is highly effective.[2,3] This approach offers a simple and direct way to prepare a variety of C-terminal modified and cyclic peptides. The initial dipeptide is attached to the backbone linker in two steps: reductive amination with amino acid esters and coupling of N-protected amino acids. After elongation of the peptide chains, the acid-labile linkage is cleaved with a TFA solution to release the expected peptides. (4-Formyl-3,5-dimethoxyphenoxy)alkyl- (Figure 1, R = R′ = OMe),[2] (4-formyl-3-methoxyphenoxy)alkyl- (Figure 1, R = OMe, R′ = H),[3] and (4-formylphenoxy)alkyl- (Figure 1, R = R′ = H)[3d] linkers have been used for the synthesis of a variety of peptide and nonpeptide compounds. The backbone linker strategy is particularly useful for preparing C-terminal modified peptides. However, this strategy suffers from serious problems when synthesizing unprotected peptides and cyclic peptides. These backbone linkers are very acid labile as a result of the electron-donating substituents on the benzylamines (di- or (trialkoxybenzyl)amines); therefore, acid-labile protecting groups for both the amine (Boc) and the acid (*tert*-Bu ester) cannot be utilized in this strategy, which restricts the usefulness of these resins in peptide

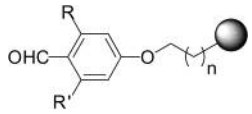


Figure 1.

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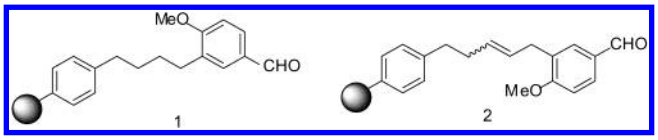
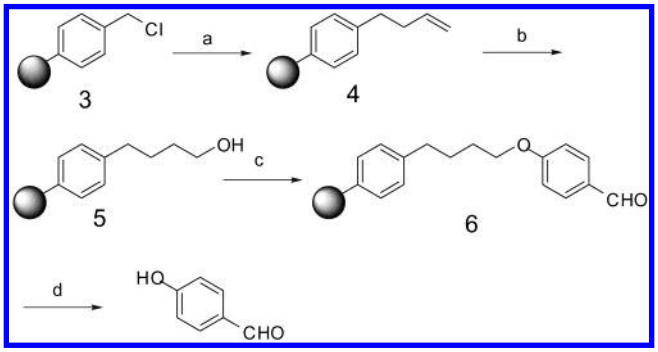
synthesis. Acid-stable Fmoc strategy can be used in peptide synthesis, but it has been found that with backbone linker-anchored dipeptides, base-promoted removal of Fmoc, even at the dipeptidyl stage, is accompanied by almost quantitative diketopiperazine formation.[4] Although diketopiperazine formation was not observed with *tert*-Bu ester protection, the requisite acid deprotection conditions for the *tert*-Bu ester causes cleavage of the peptide from the resin.

A stable aldehyde linker that is compatible with acid-labile protecting groups and can be utilized for solid-phase peptide and cyclic peptide synthesis would fill this void. Recently, a 4-alkoxybenzyl-derived linker was reported.[5] This linker allows Boc strategy, but cleavage of the peptide from the resin requires hydrofluoric acid protocols. Furthermore, the initial dipeptide has to be synthesized in solution before being attached to the linker on the solid support. Therefore, its use is restricted. Here we report two new resin-bound linkers (**1** and **2**), which are very stable during removal of common protecting groups for amines (Fmoc, Boc) and carboxylic acids (Me, All, *tert*-Bu), and the product can be cleaved from the resin with refluxing TFA.



Our initial design was a 4-alkoxybenzaldehyde linker, which was bound to polystyrene through an aryl alkyl ether. As shown in Scheme **1**, Merrifield resin **3** was treated with

Scheme 1. Synthesis of 4-Alkoxybenzaldehyde Resin[a]

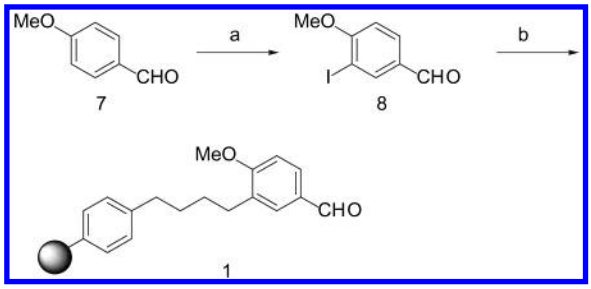


[a] Reagents and conditions: (a) allylmagnesium chloride, toluene, 60 °C; (b) 9-BBN, H_2O_2, KOH, THF; (c) 4-hydroxybenzaldehyde, DEAD, PPh_3, THF; (d) neat refluxing TFA, 3 h.

allylmagnesium chloride to afford **4**.[3a,6] Hydroboration−oxidation of **4** with 9-BBN and H_2O_2 solution afforded alcohol **5**.[3a] A Mitsunobu reaction of **5** with 4-hydroxybenzaldehyde afforded the 4-alkoxybenzaldehyde resin **6**. To determine the acid stability of the ether bond of **6**, the resin was treated with refluxing TFA for 3 h, which are conditions employed for the removal of a 4-methoxybenzyl group in amides and is a potential deprotection method for solid-phase peptide synthesis.[7] Unfortunately, cleavage of the resin was observed. One problem occasionally encountered with backbone linkers attached to polystyrene as aryl benzyl ethers is that cleavage of the entire linker can compete with C−N bond cleavage,[8] as we encountered.

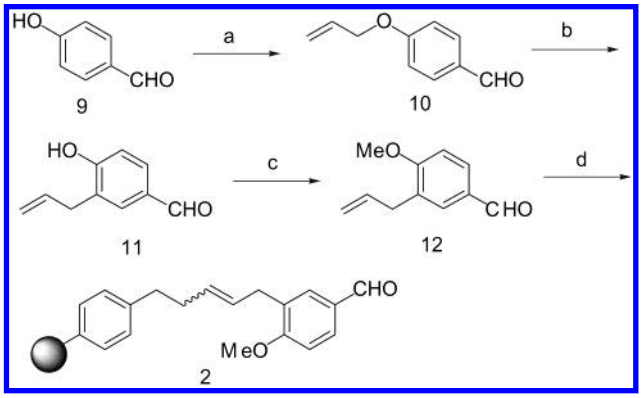
To avoid this problem, we attached 4-methoxybenzaldehyde to the resin via an alkyl chain instead of an ether linkage. As shown in Scheme **2**, the iodination of 4-methoxybenzaldehyde (**7**) with F-TEDA and I_2 in acetonitrile afforded **8**.[9] Suzuki coupling of **4** and **8**, with $Pd(PPh_3)_4$ as the catalyst and K_2CO_3 as the base,[10] afforded the 4-methoxybenzaldehyde resin **1**. To avoid the presence of "black" Pd species in **1** after the Suzuki reaction,[11] a similar linker **2** was synthesized by another approach (Scheme **3**). Allylation of 4-hydroxybenzaldehyde **9** with allyl bromide followed by Claisen rearrangement at 200 °C afforded **11**, which was treated with iodomethane using K_2CO_3 as the base

Scheme 2. Synthesis of 4-Methoxybenzaldehyde Resin **1**[a]



[a] Reagents and conditions: (a) I_2, F-TEDA, CH_3CN, 12 h, rt, 87%; (b) **4**, 9-BBN, THF, $Pd(PPh_3)_4$, 2 M Na_2CO_3, DMF, 110 °C, 3 days.

Scheme 3. Synthesis of 4-Methoxybenzaldehyde Resin **2**[a]



[a] Reagents and conditions: (a) allyl bromide, K_2CO_3, acetone, refluxing, 97%; (b) 200 °C, 5 h, 86%; (c) MeI, K_2CO_3, acetone, refluxing, 92%; (d) **4**, Grubbs Ru catalyst, CH_2Cl_2, refluxing, 24 h.

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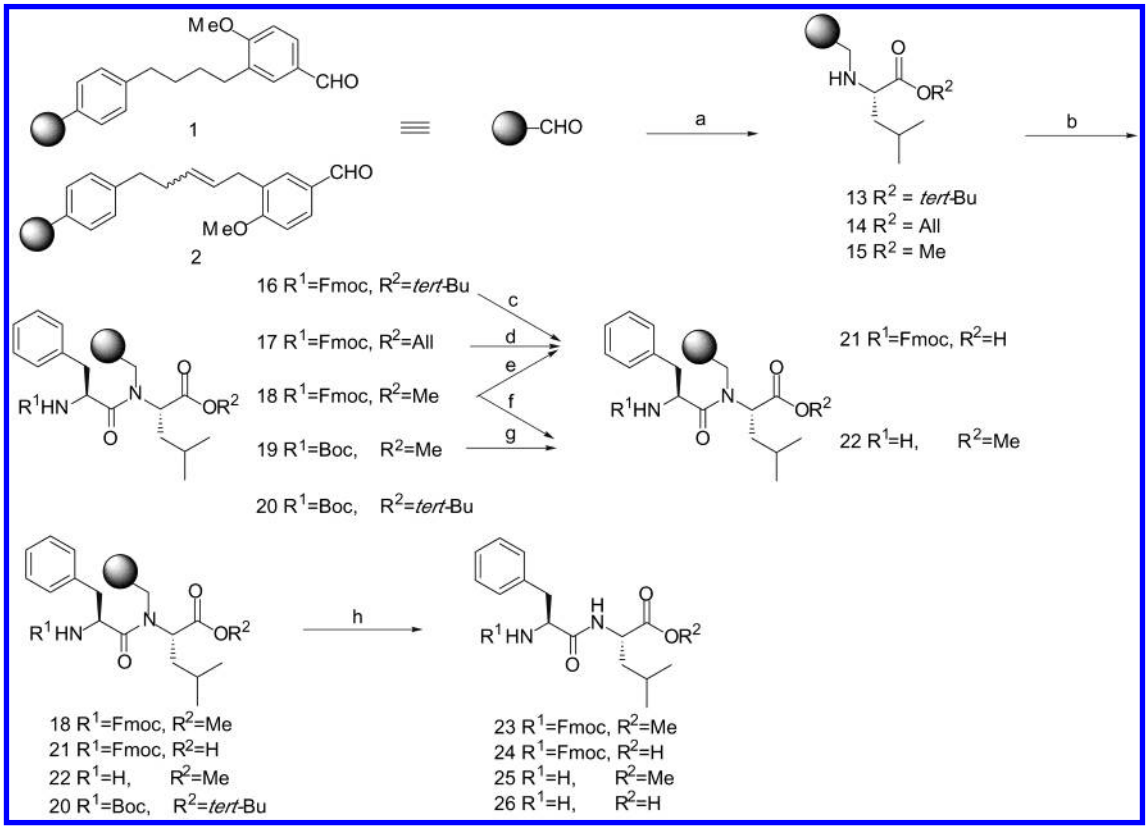
Scheme 4. Solid-Phase Synthesis of Dipeptides[a]



[a] Reagents and conditions: (a) H-Leu-OR^2·HCl (10 equiv), $NaBH(OAc)_3$ (10 equiv), 1% HOAc in DMF, 5 h; (b) R^1-Leu-OH (10 equiv), HATU (10 equiv), DIPEA (15 equiv), 9:1 CH_2Cl_2/DMF, 6 h; (c) neat TFA, rt, 1 h; (d) 37:2:1 $CHCl_3$/AcOH/NMM, $Pd(PPh_3)_4$ (5 equiv), 3 h; (e) 1 N LiOH in 1:7 H_2O/THF; (f) piperidine in DMF (1:4), 30 min; (g) 50% TFA in CH_2Cl_2, rt, 30 min; (h) neat TFA, refluxing 3 h >80% cleavage yield of all dipeptides.

to give **12**. Metathesis of **12** and **4** using the Grubbs Ru catalyst afforded resin **2**.[12]

To examine the stability of the 4-methoxybenzaldehyde resins **1** and **2** during solid-phase peptide synthesis, we synthesized dipeptide H-Phe-Leu-OH using different protecting groups. As shown in Scheme **4**, H-Leu-OR^2 was loaded onto the support by reductive amination employing $NaBH(OAc)_3$ in 1% acetic acid in DMF to afford **13**−**15**. To minimize racemization,[8,13] H-Leu-OR^2 and the reductant were premixed in 1% acetic acid in DMF followed by addition of the resin-bound aldehyde. Acylation of the resulting secondary amines **13**−**15** with R^1-Phe-OH using the highly activated azabenzotriazole-based reagent HATU as the coupling reagent provided support-bound dipeptides.[14]

To determine the stability of the 4-methoxybenzaldehyde resins **1** and **2**, five common protecting groups of amines and carboxylic acids were employed in the support-bound dipeptides **16**−**20**. The *tert*-Bu ester of **16** was removed with neat TFA for 1 h. The allyl ester of **17** was removed with $Pd(PPh_3)_4$ for 3 h. The methyl ester of **18** was removed with 1 M LiOH solution in THF for 12 h. The Fmoc group of **18** was removed with piperidine in DMF (1:4) for 30 min. The Boc group of **19** was removed with 50% TFA in methylene chloride for 30 min. After deprotection, the filtrates of every reaction were analyzed, and it was shown that no cleavage from the resin resulted. Compounds **18**, **21**, **22**, and **20** were then cleaved from the resin with refluxing TFA for 3 h to afford dipeptides **23**−**26**, respectively, in over 80% cleavage yield, based on the loading level of 4-methoxybenzaldehyde linkers **1** and **2**. These cleavage reactions also demonstrate that peptide bonds and Fmoc and methyl ester protecting groups are stable to the refluxing TFA conditions. Racemization was not observed during the reactions as determined by chiral HPLC analysis of Fmoc-Phe-Leu-OMe.

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To demonstrate that polar peptides also are stable to the refluxing TFA cleavage conditions, three other dipeptides were synthesized by the same methodology described in Scheme 4: D-Ser-L-Trp-OMe, L-Thr-L-Asn, and L-Tyr-L-Asp, from resin-bound Boc-D-Ser-L-Trp-OMe, Boc-L-Thr-L-Asn-OtBu, and Boc-L-Tyr-L-Asp(OtBu)-OtBu, respectively, were synthesized in >80% yield, based on the loading level.

Both new resins are comparable in efficiency; the differences are that higher loading levels are possible with **1**, but **2** does not contain small amounts of insoluble black Pd species.

In conclusion, we have developed two new stable 4-methoxybenzaldehyde backbone linker resins for solid-phase peptide synthesis. The 4-methoxybenzaldehyde linkers are bound to the resin through a carbon−carbon bond and are compatible with both acid- and base-labile amine and carboxylic acid protecting groups. The peptides can be removed from the solid support with refluxing TFA and conditions that do not hydrolyze Fmoc or methyl ester protecting groups and do not destroy amide bonds or common amino acid side chains.

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Supporting Information Available: Complete experimental details and product characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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