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4-Alkoxy-2-hydroxybenzaldehyde (AHB): A Versatile Aldehyde Linker for Solid-Phase Synthesis of C-Terminal Modified Peptides and Peptidomimetics†

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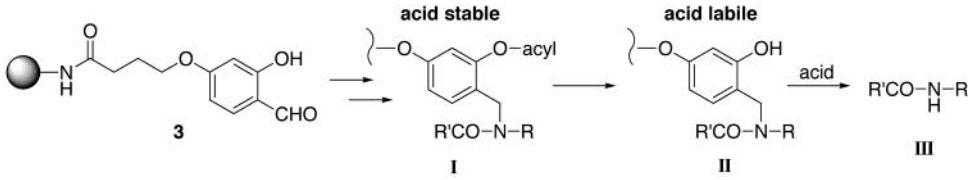
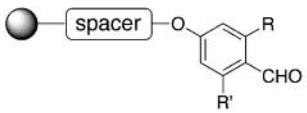
ABSTRACT



A new and versatile 4-alkoxy-2-hydroxybenzaldehyde (AHB) linker for solid-phase syntheses is described. Acylation of the polymer-bound secondary amine obtained from reductive amination of the aldehyde in the AHB linker showed good reactivity. Following acylation of the phenolic hydroxyl group, the resulting carboxamide resin was stable to treatment with 95% TFA. The *O*-acyl functional group was removed with 20% piperidine and the desired compound was cleaved from the resin by TFA treatment.

The synthesis of *C*-terminal-modified peptides and peptidomimetics on solid supports for applications to library design pose a number of synthetic challenges. Although several benzaldehyde-functionalized resins (**1**) have been reported[1] for this purpose, each of them retain some problems. For example, OMe-functionalized linkers (R = R′ = OMe or R = OMe, R′ = H) show low acid stability during assembly reactions, or without the OMe function (R = R′ = H), harsh conditions such as HF are required to cleave the desired compound from the solid support. Therefore, a more flexible methodology is needed such that the linker might be useful with both acidic and basic reaction conditions, and so that after synthesis the product can easily be cleaved from the resin. Such a modified solid support would be useful for a wide variety of applications in solid-phase synthesis. Our new aldehyde linker (**2**), which features a 4-alkoxy-2-hydroxybenzaldehyde (AHB) moiety, was designed to improve the versatility of the aldehyde linker for difficult targets that require complex synthetic procedures.



1 R= R'= OMe, R= OMe, R'= H or R= R'= H
2 R=OH, R'= H

As shown in Figure 1, acidic treatments can be employed with the AHB linker since acylation of the phenolic hydroxyl group (structure **I**) provides high acid stability to the linker for solid-phase synthesis. After the assembly is accomplished, the target compounds (**III**) can be easily released from the solid support by removal of the *O*-acyl function (structure **II**), followed by mild acid treatment such as TFA. Therefore, this new linker is compatible with the two major strategies

† Abbreviations: Ac_2O, acetic anhydride; Boc, *tert*-butoxycarbonyl; Bz, benzoyl; DIC, diisopropylcarbodiimide; DIEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; ESI, electrospray ionization; HPLC, high performance liquid chromatography; HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; Hmb, 2-hydroxy-4-methoxybenzyl; TFA, trifluoroacetic acid; TMOF, trimethyl orthoformate.

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(1) a) Songster, M. F.; Vagner, J.; Barany, G. *Lett. Pept. Sci.* **1996**, *2*, 265. (b) Boojamra, C. G.; Burrow, K. M.; Ellman, J. A. *J. Org. Chem.* **1995**, *60*, 5742. (c) Sarantakis, D.; Bickslar, J. J. *Tetrahedron Lett.* **1997**, *38*, 7325. (d) Bourne, G. T.; Meutermans, P. F.; Alewood, R. P.; McGeary, R. P.; Scanlon, M.; Watson, A. A.; Smythe, M. L. *J. Org. Chem.* **1999**, *64*, 3095.

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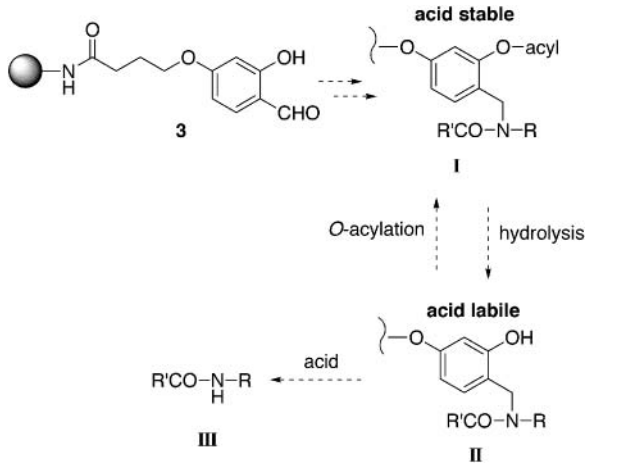


Figure 1. Structure of the AHB linker-bound resin (**1**) and illustration of switching of the acid stability of the linker.

used in peptide chemistry: Boc and Fmoc. Thus the handle is stable to piperidine treatments and after proper modification, the acylated form is stable to TFA treatment. In addition, the coupling reaction of an *N*-protected amino acid with the resin-bound secondary amine moiety could be accelerated by an O→N acyl migration mechanism similar to that observed for the Hmb protecting group.[2] In this communication, we report on the synthesis of the polystyrene-based AHB linker (**3**) and synthesis of several small molecules to demonstrate the utility of the new linker.

A representative AHB linker (**3**) was conveniently prepared from an aminomethylated polystyrene resin[3] suitable for solid-phase synthesis and 4-(4-formyl-3-hydroxyphenoxy)butyric acid.[4] Thus, 4 equiv of the acid was coupled with the amino function on the resin using DIC in the presence of an excess amount of HOBt to minimize undesired *O*-acylation to the phenolic hydroxyl group. The crude resin was treated with a mixture of 2 N NaOH and DMF (5:95) at room temperature for 1 h to hydrolyze a small amount of *O*-acylated phenol. Washing the resin with acetic acid/DMF, MeOH, and CH_2Cl_2 provided polystyrene-bound aldehyde (**3**) in quantitative yield based on the amount of amino function on the aminomethylated polystyrene.[5]

Compounds synthesized using the AHB linker are listed in Table 1. Scheme 1 shows a typical synthetic route to obtain *C*-terminal modified amino acid derivatives and peptides using the AHB linker. In method A, reductive amination of resin **3** using ethylamine or leucine *tert*-butyl ester (10 equiv) and $NaBH(OAc)_3$ in DMF/TMOF (2:1) gave the corresponding secondary amine **6**.[6] A test of the modified resin with phenylhydrazine detected no residual aldehyde. Acylation of the amino group with an *N*-benzoylated amino acid (Gly or Val, 4 equiv) via the HBTU/HOBt/DIEA method, followed by treatment with 20% piperidine/DMF to remove the *O*-acyl group afforded the carboxamide resins **7a**−**c**. The removal of the *O*-acyl function with piperidine was completed within 30 min in the case of **7a**. Cleavage of resins **7a**−**c** with 95% TFA in H_2O produced the corresponding *N*-benzoylated amino acids **4a**, **4b**, or dipeptide **4c** in high yield (Table 1, entries 1, 3, and 4). Fairly good yields of dipeptide **4c** suggested utility of the AHB linker for the backbone amide linker (BAL) strategy,[7] in which the growing peptide is anchored through a backbone nitrogen, with more flexibility. Thus, *tert*-butyl-type protecting groups (*N*-Boc and *tert*-butyl ester) can be used for *C*-terminal carboxyl and/or side chain protection combined with other orthogonal protecting groups such as allyl-type ones (*N*-Aloc and allyl ester) in the BAL strategy. Method B employed an acid treatment during the synthetic process to demonstrate the versatility of the AHB linker and gave **4a** in satisfactory yield (Table 1, entry 2). Since dimeric peptides connected through their C-terminus via hydrazine such as Biphalin[8] have been of great interest to us, synthesis of hydrazide **5** was examined (method C). Boc-protected benzylhydrazine **10** was generated

Table 1. Synthesis of *C*-Terminal Amidated Compounds Using AHB Linker[a].

entry	product	method	yield (%)
1	Bz-Gly-NHEt (**4a**)	A	97
2	Bz-Gly-NHEt (**4a**)	B	74
3	Bz-Val-NHEt (**4b**)	A	85
4	Bz-Gly-Leu-OH (**4c**)	A	78
5	Bz-Gly-$NHNH_2$ (**5**)	C	64

[a] Yields were calculated from HPLC integration using a standard curve relative to the loading level of the aldehyde linker.

(2) Johnston, T.; Quibell, M.; Owen, D.; Sheppard, R. C. *J. Chem. Soc., Chem. Commun.* **1993**, 369.

(3) Resins used were purchased from Calbiochem-Novabiochem Corp., CA.

(4) Preparation of 4-(4-formyl-2-hydroxyphenoxy)butyric acid was accomplished as follows: A mixture of ethyl 4-bromobutyrate (23.4 g, 0.12 mol), 2,4-dihydroxybenzaladehyde (16.6 g, 0.12 mol), potassium carbonate (16.6 g, 0.12 mol), and potassium iodide (2 g, 12 mmol) in DMF (60 mL) was stirred at 70 °C for 9 h and then at room-temperature overnight. Cold water (700 mL) and 6 N HCl (50 mL) were added to the reaction mixture. The resulting precipitate was filtered off and dried in vacuo. Column chromatography on silica gel, eluted with hexane/ethyl acetate (9:1), afforded 17.6 g (58%) of ethyl 4-(4-formyl-2-hydroxyphenoxy)butyrate as a white powder: mp 52−53 °C. The ethyl ester thus obtained (17.2 g, 68 mmol) was dissolved in MeOH (100 mL) and 2 N NaOH (100 mL) was added to this solution. The mixture was stirred at room temperature for 1 h. The solution was diluted with water (350 mL) and acidified with 6 N HCl (40 mL). The precipitated white powder was filtered, washed with water, and dried in vacuo over P_2O_5. Yield, 15.2 g (100%), mp 145−6 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 12.17 (s, 1H), 10.98 (s, 1H), 9.99 (s, 1H), 7.60 (d, J = 8.7 Hz, 1H), 6.56 (dd, J = 8.7, 2.3 Hz, 1H), 6.46 (d, J = 2.3 Hz, 1H), 4.03 (t, J = 6.4 Hz, 2H), 2.37 (t, J = 7.2 Hz, 2H), 1.93 (m, 2H). ESI-MS *m/z* for $C_{11}H_{12}O_5$ calcd 225 ($M + H^+$), obsd 225. Anal. Calcd for $C_{11}H_{12}O_5$: C, 58.93; H, 5.39. Found: C, 58.70; H, 5.47.

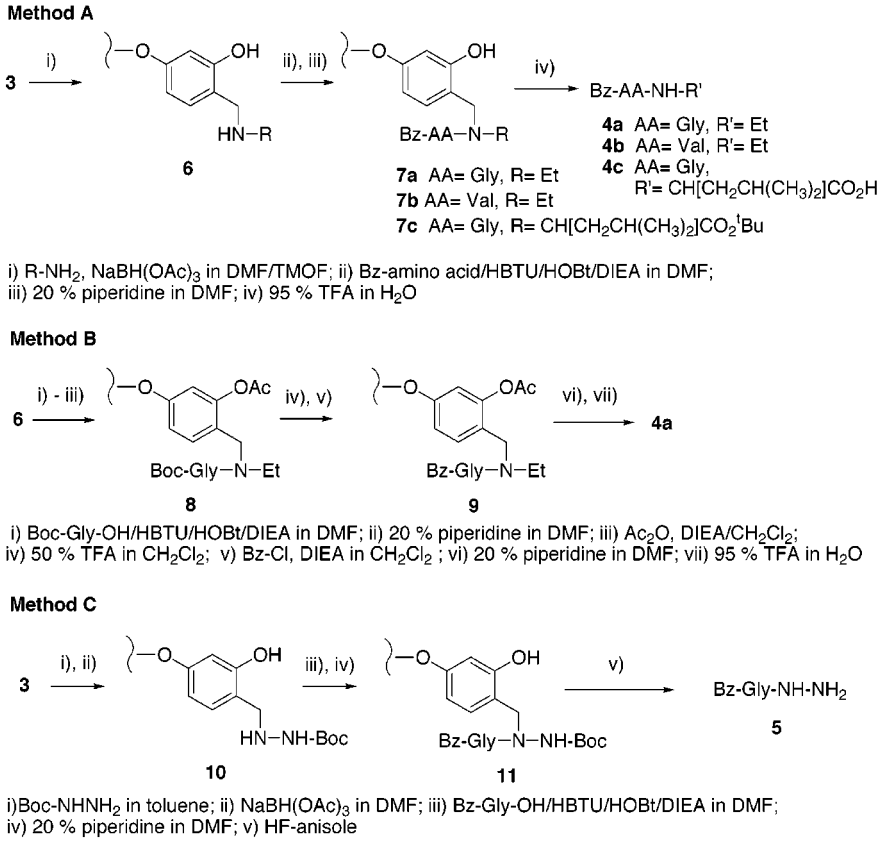
(5) The loading level of the aldehyde linker onto the aminomethylated resin was determined from the quantitative HPLC analysis of Bz-Gly-NHEt obtained by method A in Scheme 1 based on the substitution level of the aminomethylated polystyrene used.

(6) In an attempt to prepare **6**, several reaction conditions for the reductive amination were examined. $NaBH(OAc)_3$ afforded better results as a reducing agent rather than $NaBH_3CN$. As a solvent system, both DMF/TMOF and CH_2Cl_2/TMOF generally gave good results.

(7) Jensen, K. J.; Alsina, J.; Songster, M. F.; Vágner, J.; Albericio, F.; Barany, G. *J. Am. Chem. Soc.* **1998**, *120*, 5441.

(8) Misicka, A.; Lipkowski, A. W.; Horvath, R.; Davis, P.; Porreca, F.; Yamamura, H. I.; Hruby, V. J. *Life Sci.* **1997**, *60*, 1263.

Scheme 1



by hydrazone formation with Boc-$NHNH_2$ in toluene, followed by reduction with $NaBH(OAc)_3$ in DMF. Unlike alkylamide resins, hydrazide resin **10** showed an exceptional resistance against TFA treatment, so that a more severe cleavage condition (HF/anisole, 0 °C, 30 min) was required to obtain **5**. The yield of Bz-Gly-$NHNH_2$ (**11**) was acceptable, although relatively low in comparison to alkylamides (Table 1, entry 5).

The ability of the AHB linker to switch its acid stability

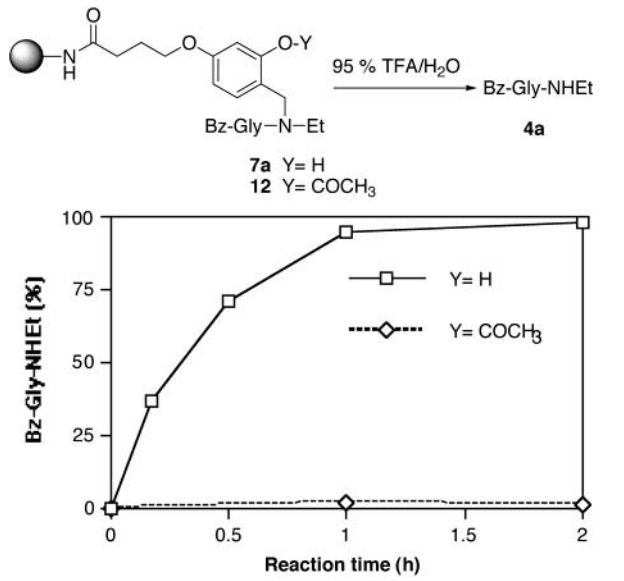


Figure 2. Comparison of acid stability.

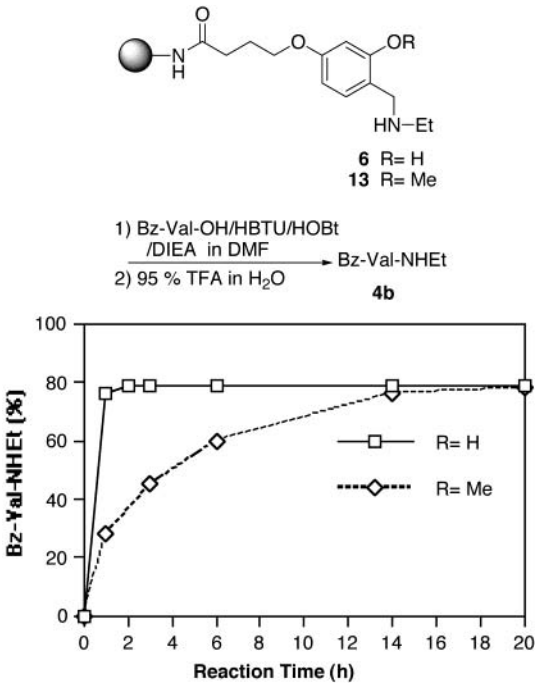


Figure 3. Comparison of the coupling rate of the amino resins.

by *O*-acylation was confirmed as shown in Figure 2. The *O*-acetylated resin **12** showed good stability against a 95% TFA in H_2O treatment, whereas Bz-Gly-NHEt (**4a**) was easily cleaved from the free OH resin **7a**. To compare the coupling reactivity of the amine resins derived from the AHB linker and the corresponding *o*-OMe benzaldehyde resin,[3] time courses of the coupling reaction with Bz-Val-OH were examined. As shown in Figure 3, the free OH resin **7b** quickly reacted to give complete reaction within 2 h. In contrast, the *O*-methylated resin **13** required a longer time, more than half a day.

In conclusion, a versatile strategy for the synthesis of *C*-terminal modified peptides and peptidomimetics on solid support has been developed using a 4-alkoxy-2-hydroxybenzaldehyde (AHB) linker. AHB linker-attached resin **3** was quantitatively prepared and shown to have advantageous properties over previously reported aldehyde linkers including stability, facile cleavage of the product from the resin, and rapid coupling reactions. The application of this strategy in the assembly of various bioactive peptides and peptidomimetics is under investigation.

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