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Wang Linker Free of Side Reactions

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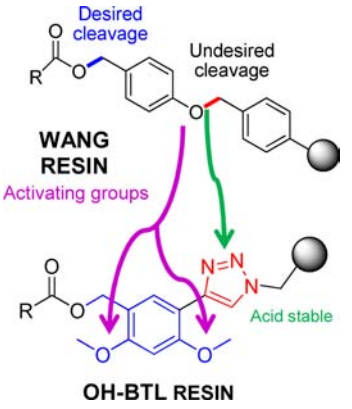
ABSTRACT



A new resin for the solid-phase synthesis of peptide acids was developed. It was based on a linker with two unique features: methoxy groups as the only activating groups of the phenyl ring and a copper(I)-catalyzed Click chemistry reaction to anchor it to the solid support. The efficiency of this new resin in solid phase peptide synthesis was compared with that of Wang resin.

Linkers for the solid phase synthesis of peptides and other organic molecules are based mostly on the presence of alkoxy phenyl moieties, the latter commonly bound to an amine-based solid support through an amide bond. At the end of the synthetic process, these resins are treated with TFA to release the target molecules, thereby rendering carboxylic acids or amides. However, this acid treatment often leads to side reactions. Thus, additional cleavages through the amide bond used to anchor the linker to the solid support and/or through the phenoxy moiety present in the linker cause the presence of side products accompanying the target.[1] These can be difficult to remove from the crude product, or worse still, they may back-alkylate the cleaved peptide at sensitive residues, such as Trp or Tyr. In addition to jeopardizing the purification, this back-alkylation decreases the final yield.[2] To circumvent this problem, here we developed some nonacid degradable linkers. These linkers are characterized by activation of the benzyl alcohol by a noncleavable electron-donating group in either the *ortho* or *para* position,[3] and/or by two phenyl rings attached by a Suzuki reaction.[4] Here we introduce a new concept of linker. In this case the linker is anchored to the solid support through a stable triazole moiety and with methoxy groups as the only activating groups of the phenyl ring (Figure 1). The efficiency of the new resin in solid phase synthesis (SPS) is compared with that of Wang resin.

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(2) The +107 peak corresponding to the Wang linker is a purification nightmare for most peptides produced using this support.

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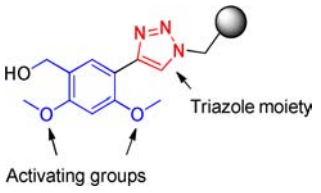


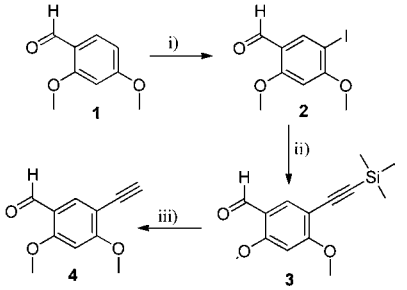
Figure 1. Linker concept.

The copper-catalyzed azide−alkyne cycloaddition (CuAAC) has been extensively used in SPS to obtain cyclic peptides,[5] triazolyl aminoacyl (peptidyl) penicillins,[6] and aldehyde-functionalized resins.[7] For efficient and stable coupling of the linker to a solid support, this reaction requires an alkyne- and an azide-functionalized resin as the solid support.[8]

First, 5-ethynyl-2,4-dimethoxybenzaldehyde (**4**) was synthesized from commercially available 2,4-dimethoxybenzaldehyde (**1**) (Scheme 1), which was iodated with iodine monochloride at C5 of **1**.[9] The alkyne moiety was introduced by a Sonogashira coupling reaction,[10] exchanging the iodine atom by TMSA, to obtain **3**. Subsequent deprotection gave the intermediate **4** in an overall yield of 28%.

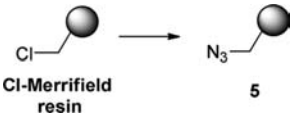
To generate the Merrifield azide resin **5**, Cl−Merrifield resin (0.7 mmol Cl/g) was reacted with sodium azide in DMF (Scheme 2) following a previous reported method with slight modifications (conversion was carried at room temperature rather than at 60 °C).[11] The reduction/ninhydrin test was used to monitor the incorporation of azide function on the resin.[12] Treatment with the standard PPh_3, ninhydrin, pyridine, and phenol solutions followed by warming gave a positive result (solution turned dark purple). This step was also monitored using IR spectroscopy to corroborate the successful conversion of **5** (ν_{N_3} = 2092 cm^{-1}).

Scheme 1. Synthesis of 5-Ethynyl-2,4-dimethoxybenzaldehyde (**4**)[a]



[a] (i) ICl (1.2 equiv), MeOH, 3 h, rt; (ii) $Pd(PPh_3)_4$ (0.03 equiv), CuI (0.005 equiv), Piperidine (1.9 equiv), TMSA (1.2 equiv), THF, 1 h, rt; (iii) K_2CO_3 (2 equiv), DCM/MeOH (9:1), 1 h, rt.

Scheme 2. Chloro Merrifield Resin Conversion[a]



[a] NaN_3 (3 equiv), DMF, 24 h, rt.

Intermediate **4** was loaded onto the Merrifield azide resin **5** (Scheme 3) via CuAAC[13] to render CHO-BTL resin. This reaction was carried out in ACN, ascorbate of sodium, 2,6-lutidine, DIEA, and DMF as solvent for 6 h at room temperature. If only catalytic amounts of CuBr are used, reaction times should increase. The progress of CuAAC was monitored using IR spectroscopy, through the loss of the azide band and the appearance of new vibrational modes at 1673 cm^{-1} and 1546 cm^{-1}, indicative of the carbonyl group and the alkene of the triazol moiety, respectively.

The OH-BTL resin was finally obtained by reducing CHO-BTL resin using $NaBH_4$ in THF/MeOH.

The OH-BTL resin was characterized by IR spectrometry, and its stability was evaluated under standard acid conditions by treatment with the cleavage cocktail [TFA/TIS/H_2O (95:2.5:2.5)] during 1, 2, 3, and 24 h. Filtrates after each treatment were analyzed by HPLC and HPLC-MS. No cleavage from the resin was detected. Also, no change in the IR spectra of any of the residual resins was detected. Once the stability of the linker anchored to the resin was validated, the scope and limitations of this new resin in comparison with a standard Wang resin were examined. Thus, three model peptides were synthesized by the Fmoc/^{t}Bu strategy: Leu-enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH) **6**, RGD pentapetide (H-Arg-Gly-Asp-Gly-Trp-OH) **7**, and Indolicidin (H-Ile-Leu-Pro-Trp-Lys-Trp-Pro-Trp-Trp-Pro-Trp-Arg-Arg-OH) **8**.

The first Fmoc-amino acid was incorporated into both resins using the symmetrical anhydride in the presence of catalytic amounts of DMAP, while DIPCDI-OxymaPure was used for the rest of the residues.[14] The symmetrical anhydride was a satisfactory method for introducing the first amino acid on the OH-BTL resin (Table 1), as demonstrated by means of UV analysis of the

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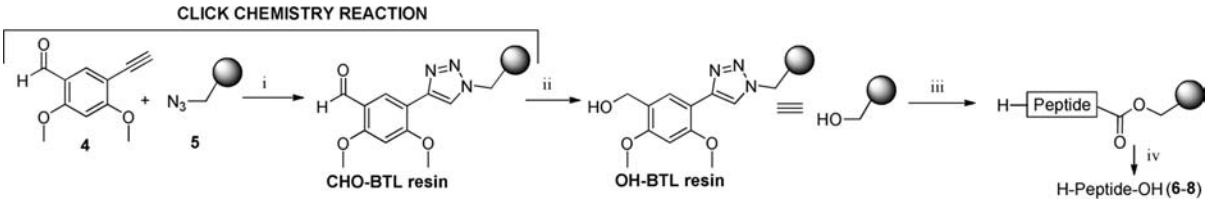
Scheme 3. Click Chemistry Reaction on Solid Support and Solid Phase Peptide Synthesis (SPPS)[a]



[a] (i) CuBr (1 equiv) in ACN, Na ascorbate (1 equiv), 2,6-lutidine (10 equiv), DIEA (10 equiv), DMF, 6 h, rt; (ii) $NaBH_4$ (3 equiv), THF/MeOH (1:1), 1 h, rt; (iii) SPPS with Fmoc/^{t}Bu strategy; (iv) cleavage.

Table 1. Results of Resin Substitution

resin	peptide	yields (%)[a,b]
OH-BTL	**6**	92, 100
	7	82, 42
	8	80, 32
Wang	**6**	100, 80
	7	78, 36
	8	70, 23

[a] Yields calculated by Fmoc UV determination taking as initial loading 0.9 mmol/g for Wang resin and 0.6 mmol/g, for OH-BTL resin. [b] Yield determined by AAA of hydrolyzed peptides attached to resin (HCl/propionic acid (1:1), PhOH).

Table 2. SPPS Results

resins	cleavage[a]	peptide	% yield[b]	% purity[c]	%[d]
OH-BTL	I	6	86	98.2	–
	II		100	97.4	–
	I	7	83	94.0	–
	II		83	93.9	–
	I	8	44	95.3	–
	II		48	89.8	–
Wang	II	6	80	97.4	0.8
	II	7	61	77.5	15.8
	II	8	35	74.7	11.1

[a] Cleavage conditions: I, TFA/DCM/TIS/H_2O (50:45:2.5:2.5); II, TFA/TIS/H_2O (95:2.5:2.5). [b] Yields were calculated by weight, taking as a reference the amount of peptide obtained when the resin was cleaved. [c] Purity measured by HPLC at 220 nm. [d] Byproduct percent corresponding to $(M + 107)^+$, detected by HPLC at 220 nm.

piperidine–dibenzofulvene adduct.[15] The peptide content of the final peptide resin calculated by amino acid analysis (AAA) was shown to be superior for the OH-BTL resin for all three peptides (Table 1).

Two cleavage treatments at different acid concentrations (I and II, Table 2) were tested to release peptides **6**–**8** from the resins. Both cocktails rendered the three peptides with similar yields. The purities of the peptides were slightly higher with the less acidic cleavage cocktail (I) (Table 2). Use of a more diluted cleavage cocktail (< 10% of TFA) was not efficient as expected (data not shown).

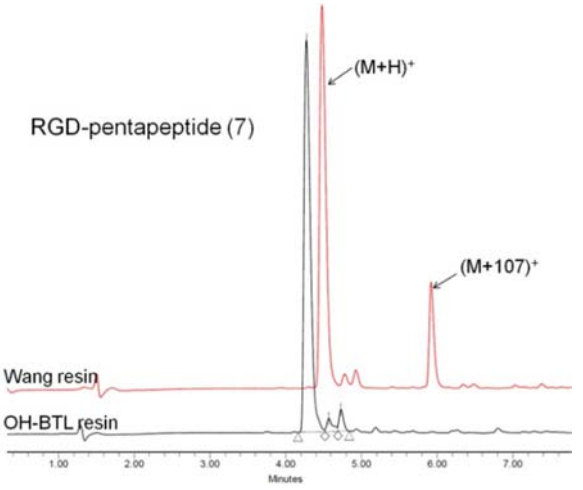


Figure 2. HPLC profile comparison of peptide **7** synthesized on OH-BTL or Wang resin.

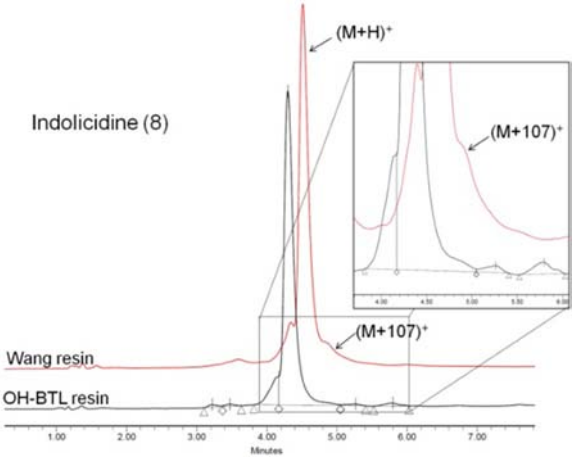


Figure 3. HPLC profile comparison of peptide **8** synthesized on OH-BTL or Wang resin.

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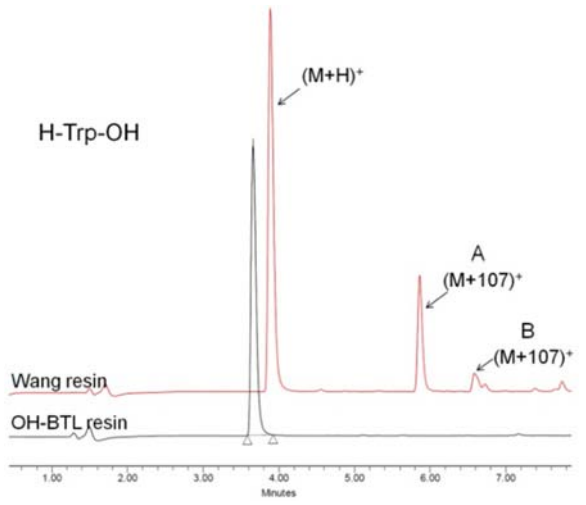
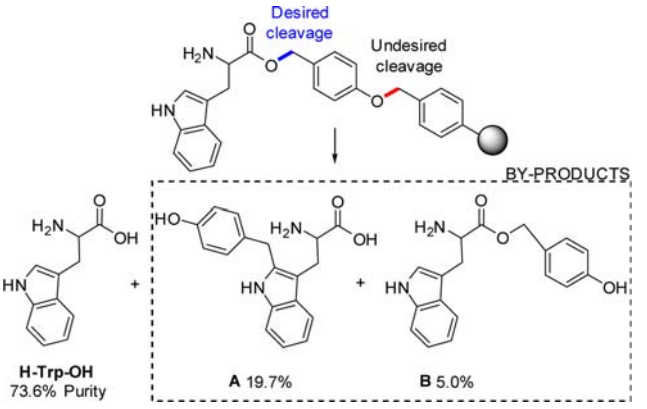


Figure 4. HPLC profiles comparison of H-Trp-OH synthesized on OH-BTL or Wang resin. "A" corresponds to alkylated Trp derivative; "B" corresponds to undesired cleavage product.

For all syntheses, the yields with OH-BTL resin were higher than those with Wang resin. These two resins showed similar purities for peptide **6**. However, even for this peptide, which did not contain Trp, the crude product obtained with the Wang resin showed a peak of $(M + 107)^+$ (0.8%), as determined by HPLC analysis. This peak was probably caused by the 4-hydroxymethyl *p*-hydroxybenzyl ester analog (see Supporting Information). In the case of the RGD pentapeptide **7** and indolicidin **8**, the purities on OH-BTL were higher than those on Wang resin. The HPLC and HPLC-MS analysis show that impurities on the latter resin were mainly due to Trp alkylation (Table 2), in both cases showing the peak corresponding to $(M + 107)^+$ (Figures 2, 3).

In order to prove the absence of side reactions on OH-BTL resin, the Fmoc-Trp-OH was coupled on both resins (OH-BTL and Wang). Fmoc removal and cleavage under the acid conditions II [TFA/TIS/H_2O (95:2.5:2.5)] in both resins rendered H-Trp-OH at 3.6 min with purities of 100 and 73.6% for OH-BTL and Wang resin respectively (Figure 4). In Wang resin, two peaks at 5.6 and 6.3 min corresponded to a back-alkylated product (peak A, Figure 4) and to an ester caused by the undesired cleavage product respectively (peak B, Figure 4) (Scheme 4).

Scheme 4. Cleavage of H-Trp-OH on Wang Resin[a]



[a] Cleavage conditions: II, TFA/TIS/H_2O (95:2.5:2.5). Side products: (A) alkylated Trp derivative; (B) undesired cleavage product.

In conclusion, here we developed a new acid-labile resin for the preparation of acid peptides with two unique features: methoxy groups as the only activating groups of the phenyl ring and a CuAAC to anchor the linker to the solid support. OH-BTL-resin was more acid-stable and more efficient for SPPS than Wang resin. Using this new resin, three model peptides were obtained with higher yields and purities when compared with those achieved with Wang resin. The final proof of concept of the superiority of OH-BTL-resin was obtained after cleavage of Trp from resins. The same concept, methoxy groups for activating and CuAAC for anchoring, is being extended to other alkoxybenzyl resins such as Rink and BAL.

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Supporting Information Available. Additional information regarding the synthesis and characterization of organic compounds by NMR, IR, HPLC, HPLC-MS, and HRMS. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.