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Acid-Triggered Colorimetric Hydrophobic Benzyl Alcohols for Soluble Tag-Assisted Liquid-Phase Synthesis

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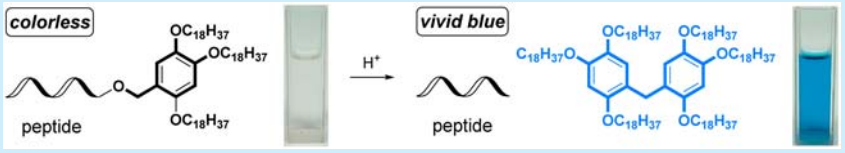
Supporting Information



ABSTRACT: Simple screening of acid-triggered reactions of methoxybenzyl alcohols led to the development of a novel colorimetric hydrophobic benzyl alcohol (HBA) tag. HBA tag-3 (**14**) retained high solubility in less polar solvents and excellent precipitation properties in polar solvents. Our routine procedure for tag-assisted liquid phase peptide synthesis was applied using HBA tag-3 (**14**), and an effective synthesis of β-sheet breaker peptide iAβ5 (**4**) was achieved. The tagged peptides showed a vivid blue color under acidic conditions both on TLC plates and in solution, enabling quantitative assay.

The trade-off between solid phase and liquid phase synthesis launched the field of chemical synthesis using soluble polymer supports.[1] Soluble polymers may be used to support reactants or catalysts, allowing a reaction to be carried out under homogeneous conditions. When the reaction is complete, the supported product or catalyst is simply separated by filtration or centrifugation after phase perturbation, which induces polymer precipitation. The most general way to achieve phase perturbation is a change of solvent to one in which the polymer is not soluble. A key criterion for the design and successful implementation of such chemical synthesis using soluble polymer supports is high control of the polymer solubility. High solubility is required before and during the reaction, but the polymer must be entirely insoluble after the phase perturbation; otherwise, there would be significant loss over multistep or repeating processes. This is especially true for peptide synthesis. For example, only 35% of a hexapeptide is recovered after five cycles of deprotection and coupling, even if the precipitate yield at each step is 90%.[2]

Polyethylene glycols (PEGs) were among the first soluble polymer supports to be used in chemical synthesis, and they are central to many applications in the field.[3] PEGs have up to two terminal hydroxy groups with molecular weights of less than 20000, enabling loadings of at least 0.10 mmol/g. In general, PEGs are soluble in typical polar solvents and insoluble in typical less polar solvents. Therefore, a reaction of a supported reactant or catalyst can be performed under homogeneous conditions in a polar solvent, and the polymer is precipitated by the addition of a less polar solvent. However, as for peptide synthesis, the opposite solubility is preferable because both excess amino acids and coupling reagents are generally polar and can be rinsed away effectively by washing with polar solvents.

We have been developing chemical synthesis using hydrophobic benzyl alcohols (HBAs) as soluble tags.[4] Our HBA tags, which have one hydroxy group, have a molecular weight of 757.33, enabling loadings of 1.3 mmol/g (Figure 1, above). The

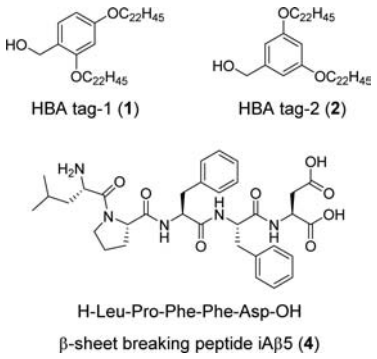


Figure 1. Structures of HBA tags and β-sheet breaker peptide iAβ5 (**4**).

tags are soluble in less polar solvents, including toluene, dichloromethane, and tetrahydrofuran, but insoluble in polar solvents, including *N*,*N*-dimethylformamide, methanol, and acetonitrile. HBA tags can be prepared easily from commercially available starting materials in two steps and can be obtained in

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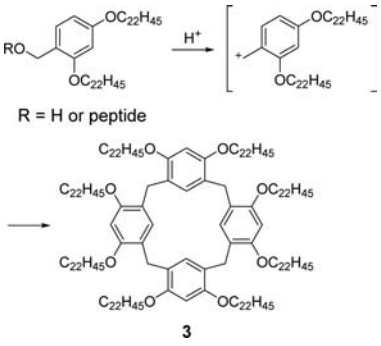
excellent precipitation yield through phase perturbation. We have used HBA tags to support amino acids, leading to practical production of several bioactive peptides. It should also be noted that the substitution pattern on the aromatic ring can significantly affect the properties of the HBA tags. For example, the ester bond of HBA tag-1 (**1**) can be cleaved using 1% trifluoroacetic acid, while that of HBA tag-2 (**2**) is much more acid-resistant, enabling the use of Boc chemistry.[5]

Functional HBA tags are a unique new item in the growing toolbox of chemical synthesis using soluble tags. In particular, the application of colorimetric properties is promising for the detection of tagged reactants or catalysts. Although it may seem like a technical issue, colorimetric properties are also highly useful in TLC analysis. However, the design of such colorimetric HBA tags must be creative, because they are required to show easily distinguishable color with minimal structural modification; otherwise, the solubility and precipitation properties of the HBA tags may be affected significantly.

During a previous study, we found that HBA tag-1 (**1**) and the tagged peptides showed a vivid purple color under acidic conditions both in solution and on a TLC plate. This was thought to be due to the formation of a resorcinarene derivative (**3**), probably triggered by the generation of benzyl cations (Scheme 1). The possible mechanism is favorable, because the color

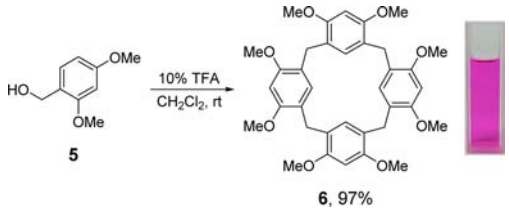
Scheme 1. Acid-Triggered Reaction of HBA Tag-1 (1) and Tagged Peptide



reaction occurs regardless of sequence. Although it was helpful in TLC analysis, quantitative assay in solution remained challenging because the resorcinarene derivative (**3**) was not sufficiently soluble, resulting in a vivid purple turbid mixture (Figure S1 in Supporting Information). We expected that HBA tags with a different substituted pattern on the aromatic ring might form more soluble cyclic oligomers under acidic conditions, to be used for quantitative assay in solution. Described herein is our serendipitous development of a new colorimetric HBA tag and its application in the synthesis of β-sheet breaker peptide iAβ5 (**4**) (Figure 1, below).

The work began with the screening of acid-triggered reactions of methoxybenzyl alcohols as models for HBA tags. The potential candidate would show a vivid color, ideally after a short period of time. Methoxybenzyl alcohols were treated with 10% trifluoroacetic acid in dichloromethane at room temperature, and the reaction was quenched after 10 min (see Supporting Information for experimental details). Initially, 2,4-dimethoxybenzyl alcohol (**5**) was selected to mimic HBA tag-1 (**1**), to confirm that methoxybenzyl alcohols had similar reactivity to the HBA tags (Scheme 2). As expected, the reaction proceeded smoothly to form a resorcinarene derivative (**6**)[6] in 97% yield, and the reaction solution was a vivid purple color, similar to that of HBA

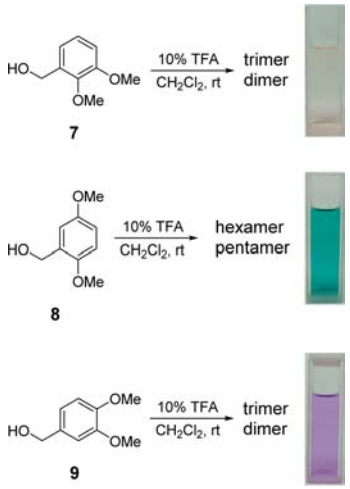
Scheme 2. Acid-Triggered Reaction of 2,4-Dimethoxybenzyl Alcohol (5). Picture of Crude Reaction Mixture Was Taken at 30 mM Concentration



tag-1 (**1**) (Figure S2 for UV−vis and MS spectra). This showed that new HBA tags could be simply designed based on the corresponding methoxybenzyl alcohols.

With this result in hand, we tested three dimethoxybenzyl alcohols (**7**−**9**) (Scheme 3). Because HBA tag-2 (**2**) was much

Scheme 3. Acid-Triggered Reaction of Dimethoxybenzyl Alcohols (7−9). The Products Described Are the Two Main Oligomers. Pictures of Crude Reaction Mixtures Were Taken at 30 mM Concentrations



less reactive to acid and did not show any color reaction, we expected that at least an *ortho*- or *para*-alkoxy substituent would be required. Unfortunately, although some showed vivid colors, all reactions were slower than that of 2,4-dimethoxybenzyl alcohol (**5**), and gave oligomeric mixtures (see Figures S3−S5 for UV−vis and MS spectra). In the case of 2,3-dimethoxybenzyl alcohol (**7**), almost no conversion was observed and the reaction solution remained colorless. However, 2,5-dimethoxybenzyl alcohol (**8**) gave pilararene derivatives,[7] mainly hexamer and pentamer, with green color. In contrast, 3,4-dimethoxybenzyl alcohol (**9**) mainly gave dimer, showing purple color.

On the basis of these observations, we then focused on trimethoxybenzyl alcohols. Because 2,4-dimethoxybenzyl alcohol (**5**) had been the most promising methoxybenzyl alcohol so far, 2,4,5-trimethoxybenzyl alcohol (**10**) was chosen. Surprisingly, the reaction was completed in 1 min, forming a dimer (**11**)[8] in 97% yield, and the reaction solution was a vivid blue color (λ_{max} = 612 nm) (Scheme 4; see Figure S6 for UV−vis and MS spectra). Although the exact mechanism was unclear, the reaction was thought to have occurred through *ipso*-substitution by a dealkylative coupling pathway.[9] Such a rare pathway is known to be induced by electron transfer using either a one-electron oxidant or anodic oxidation. However, the electron

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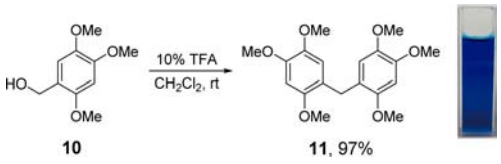
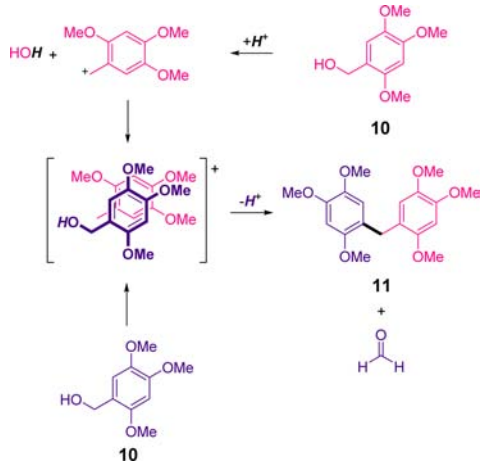
Scheme 4. Acid-Triggered Reaction of 2,4,5-Trimethoxybenzyl Alcohol (10). Picture of Crude Reaction Mixture Was Taken at 30 mM Concentration



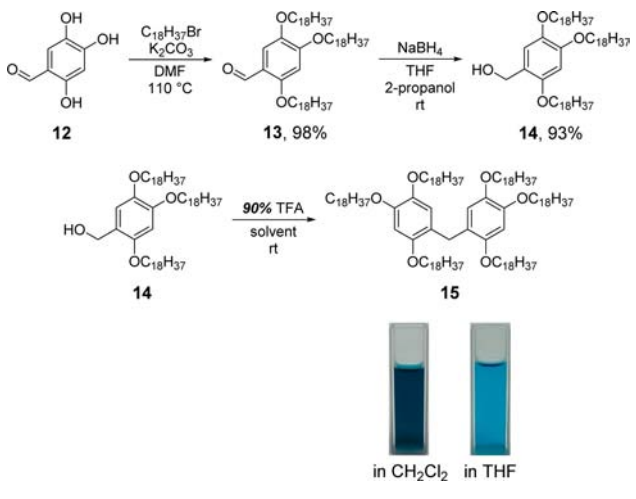
transfer process is not likely to be involved in acid-triggered dealkylative coupling, which probably takes place via a retro Friedel–Crafts mechanism prompted by cation-π interactions to generate formaldehyde (Scheme 5).

Scheme 5. Plausible Mechanism of Acid-Triggered Reaction of 2,4,5-Trimethoxybenzyl Alcohol (10)



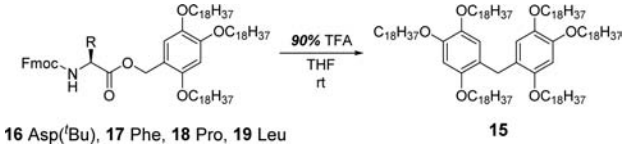
Encouraged by this finding, we then sought to prepare a new HBA tag with 2,4,5-alkoxy substituents (Scheme 6, above). Thus, 2,4,5-trihydroxybenzaldehyde (**12**) was alkylated with 1-bromooctadecane to give the hydrophobic benzaldehyde (**13**), which was then reduced with sodium borohydride to afford the desired HBA tag-3 (**14**) in 91% over two steps (see Supporting Information for experimental details). To our satisfaction, the acid-triggered reaction of HBA tag-3 (**14**) smoothly proceeded

Scheme 6. Preparation of HBA tag 14, and Acid-Triggered Reaction. Pictures Were Taken at 1 mM Concentrations



to form a dimer (**15**) in 10 min, and the reaction solution showed a vivid blue color (λ_{max} = 612 nm) similar to that of 2,4,5-trimethoxybenzyl alcohol (**10**), (Scheme 6, below; see Figure S7 for UV–vis spectrum). We also found that the acid-triggered reaction of the HBA tag-3 (**14**) completed in 1 min when tetrahydrofuran was used instead of dichloromethane, showing a vivid lighter blue color (Scheme 6, below; see Figure S7 for UV–vis and MS spectra). The vivid blue color was stable under an acidic condition, which was maintained at least 24 h (Figure S8). In these cases, a stronger acidic condition of 90% trifluoroacetic acid was effective for rapid color reaction. Notably, the dimer (**15**) was much more soluble in several solvents than the resorcinarene derivative (**3**), which is preferable for quantitative assay in solution (Figure S9). However, in practice the formation of the dimer (**15**) must occur from the ester bond of HBA tag-3 (**14**). Therefore, we coupled the constituent amino acids of β-sheet breaker peptide iAβ5 (**4**), and then attempted acid-triggered reactions (Scheme 7). Gratifyingly, all esters (**16**–**19**)

Scheme 7. Acid-Triggered Reaction of the Esters of HBA Tag-3 (16–19)



showed a vivid blue color (λ_{max} = 612 nm) under acidic conditions in 1 min, suggesting that dealkylative coupling probably took place after cleavage of the ester (Scheme 7; see Figures S10–S13 for UV–vis spectra). Additionally, when it comes to quantitative assay, the calibration curves must be important. We then measured UV–vis spectra at various concentrations to prepare the calibration curves for each ester. In this case, the reaction time of 10 min was employed to reduce measurement errors. We confirmed that the calibration curves for all esters (**16**–**19**) displayed good linearity (Figure 2).

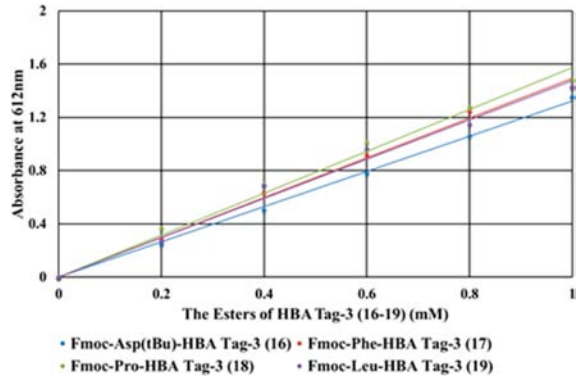


Figure 2. Calibration curves of acid-triggered colorimetric reactions of the esters of HBA tag-3 (**16**–**19**).

Finally, we used HBA tag-3 (**14**) for the synthesis of β-sheet breaker peptide iAβ5 (**4**). Tag-assisted liquid phase peptide synthesis was achieved based on a routine procedure (Scheme 8; see SI for experimental details). We found that HBA tag-3 (**14**) retained high solubility in less polar solvents and excellent precipitation properties in polar solvents, enabling synthesis of the tagged peptide (**20**) in 88% yield over nine steps (see Figure S14 for purity). The final global deprotection proceeded

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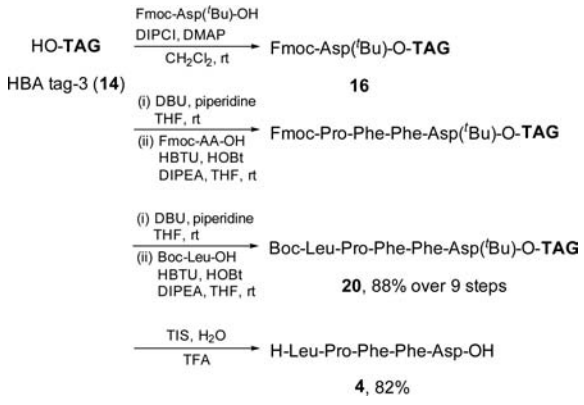
Scheme 8. Synthesis of Peptide 4 Using HBA Tag-3 (14)



smoothly to give β-sheet breaker peptide iAβ5 (**4**) in 82% yield (see Figure S15 for purity).

We prepared calibration curves for each tagged peptide and confirmed that they are usefully linear at relatively high concentration (Figure S16 in SI). Using the calibration curves, we attempted a quantitative assay using acid-triggered colorimetric reactions. To improve the accuracy of the assay, we took a 100 μL sample from each reaction solution, which was quickly passed through a short column to remove excess amino acids and reagents before the acid-triggered colorimetric reaction (see SI for experimental details). For example, the yield of the third coupling of Fmoc-Phe-OH to H-Phe-Asp(^{t}Bu)-O-**TAG** was 97%, which was estimated at 96% using UV−vis spectroscopy.

In conclusion, a novel colorimetric HBA tag has been successfully added to the growing toolbox of chemical synthesis using soluble tags. HBA tag-3 (**14**), which retains high solubility in less polar solvents and excellent precipitation properties in polar solvents, was developed through simple screening of acid-triggered reactions of methoxybenzyl alcohols as models. We demonstrated an effective synthesis of β-sheet breaker peptide iAβ5 (**4**) using HBA tag-3 (**14**) by a routine procedure. The tagged peptides showed a vivid blue color under acidic conditions both on TLC plates and in solution, enabling quantitative assay. Future work will be directed toward understanding the exact mechanism of the color reaction.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02057.

Additional figures, general, experimental, and spectra information. (PDF)

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Notes

The authors declare no competing financial interest.

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