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The 4-*tert*-butylphenyl group as a simple tag for solution phase synthesis

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Abstract—A solution phase synthesis strategy was investigated using 4-*tert*-butylphenyl group as the tag and a beta-cyclodextrin column as the affinity chromatographic support for the isolation of compounds containing the tag. It was found that compounds containing the tag have significantly longer retention times on the beta-cyclodextrin column than those compounds that do not have such a tag. The tag is chemically inert and can be introduced onto and removed from target compounds readily. This solution phase synthesis method was applied to the synthesis of some simple amino acid derivatives.

Since the practical synthesis of organic compounds is limited not only by the yield of specific reactions but also by the ability to isolate product efficiently, various strategies to facilitate product isolation have been developed in the past. Among them, syntheses using insoluble polymeric support (solid phase synthesis) are widely used for peptide[1] and oligo-nucleotide synthesis.[2] Solid phase synthesis has also been investigated for the preparation of other type of compounds such as polysaccharides[3] and small organic molecules in connection with high throughput synthesis.[4] While it proves to be an effective strategy, it has its limitations. For example, many organic reactions need to be re-optimized for solid phase synthesis and some do not work well on solid support. Reactions on solid supports are also more difficult to monitor. Moreover, it is impossible to purify reaction intermediates in solid phase synthesis. An alternative strategy to facilitate product isolation involves solution phase synthesis onto a soluble tag. In this strategy, a facile isolation process is made possible due to certain properties of the tag. The ideal tag should also be inert in the reaction conditions. Examples of this tag strategy include synthesis using soluble polymers such as polyethylene glycol.[5,6] More recently, small fluorous tags that have high affinity toward fluorous silica gel have attracted significant attention in organic chemistry.[7] These fluorous tags have been applied successfully to many synthetic reactions.[8] In this article, we would like to report a new tagging system based on the interaction of beta-cyclodextrin and the 4-*tert*-butylphenyl group. Concurrently, Dandapani et al. introduce 1-adamantyl as a tagging group for a similar application.[9]

Beta-cyclodextrin, a cyclic oligosaccharide, has been well studied in host–guest chemistry and separation science.[10–12] It is known to bind 4-*tert*-butylphenol with an association constant of $3.6 \times 10^4\,M^{-1}$ in water.[13] The strong interaction is hydrophobic in nature and results from the structural complimentary of 4-*tert*-butylphenol to the hydrophobic cavity of beta-cyclodextrin. We postulate that the beta-cyclodextrin column, widely used for chiral separation,[14] may provide an efficient mechanism of separating compounds containing 4-*tert*-butylphenyl group from compounds that do not have such a functional group based on this mechanism. Since 4-*tert*-butylphenyl is also inert in many organic reactions, it could prove to be a useful tag for solution phase synthesis.

For the successful application of tag directed synthesis, it is important that functional groups attached to the tag do not impact its separation mechanism significantly. In this particular case, since the intended isolation mechanism of this tag involves chromatographic

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separation using the cyclodextrin column, it is important that the retention times of tagged compounds do not differ significantly from each other but differ significantly from those of non-tagged compounds. Therefore, chromatographic behaviors of a number of compounds that include both tagged and non-tagged compounds with different polarities were studied.

Using a commercial beta-cyclodextrin column from Advanced Separation Technologies Inc., the retention time of 4-*tert*-butylphenol was measured under reversed phase conditions along with the retention times of a number of other compounds that do not contain 4-*tert*-butylphenyl group. It is clear that the retention time of 4-*tert*-butylphenol is significantly longer than the retention times of these compounds that do not contain 4-*tert*-butylphenyl group, despite many of which are more hydrophobic than 4-*tert*-butylphenol (Table 1). The observed selectivity is quite impressive, as three compounds [Tyr(*t*Bu)-O*t*Bu, Fmoc-Tyr(*t*Bu)-OH, and Ac-Tyr(*t*Bu)-O*t*Bu] that contain 4-*tert*-butoxyphenyl group were not strongly retained, although 4-*tert*-butoxyphenyl is somewhat similar to 4-*tert*-butylphenyl group. Under the gradient elution conditions, the retention times of these compounds that do not contain 4-*tert*-butylphenyl group also fall into a narrow range.

Table 1. Retention times of representative compounds on a beta-cyclodextrin column

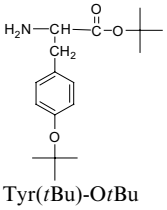
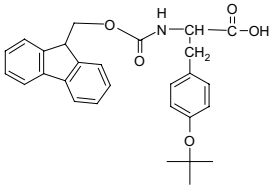
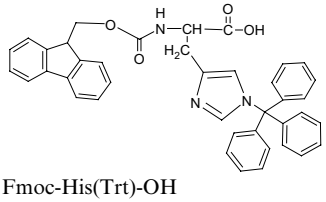
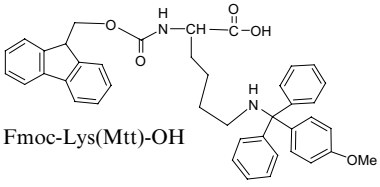
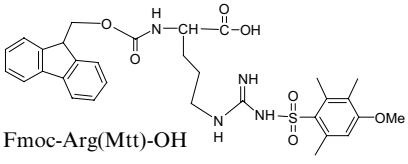
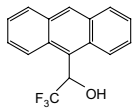
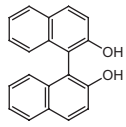
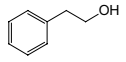
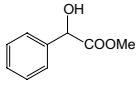
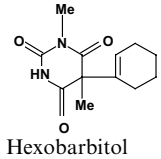
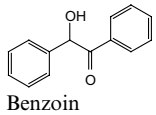
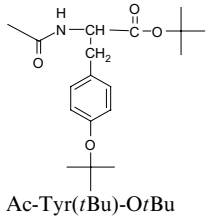
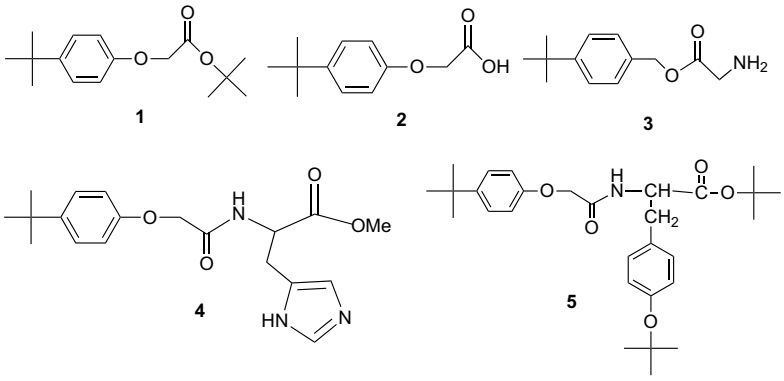
Compounds	Retention time (min)
4-*tert*-Butylphenol	14.7
 Tyr(*t*Bu)-O*t*Bu	5.1
Fmoc-Phe-OH	5.3
Fmoc-Ile-OH	4.1
 Fmoc-Tyr(*t*Bu)-OH	6.5
 Fmoc-His(Trt)-OH	4.7
 Fmoc-Lys(Mtt)-OH	4.4
 Fmoc-Arg(Mtt)-OH	4.1

Table 1 (*continued*)

Compounds	Retention time (min)
 2,2,2-Trifluoro-1-(9-anthryl)ethanol	4.2
 1,1′-Bi-2-naphthol	6.3
 Phenethyl alcohol	4.1
 Methyl mandelate	3.3
Warfarin	6.1
 Hexobarbitol	3.5
 Benzoin	4.1
Benzyl alcohol	4.2
 Ac-Tyr(*t*Bu)-O*t*Bu	5.1

Mobile phase: a linear gradient from 50% methanol to 100% methanol in 0.5% TFA/water over 1 h. Flow rate: 1 mL/min; column size: 25 cm × 4.6 mm; dead time = 3.1 min.

The effectiveness of 4-*tert*-butylphenyl as a tagging group for chromatographic separation is then studied. For this purpose, several compounds (Scheme 1) were tagged with 4-*tert*-butylphenyl group by following well-known procedures and their retention times were determined using the same column under two mobile phase conditions, one with trifluoroacetic acid (TFA) (mobile phase A) and one without TFA (mobile phase B) in it. As seen from Table 2, with TFA in the mobile phase, retention times of the tagged compounds (**1**–**5**)



Scheme 1. Tagged compounds for evaluations.

Table 2. Effectiveness of 4-*tert*-butylphenyl group as a tag for column separation using a beta-cyclodextrin column

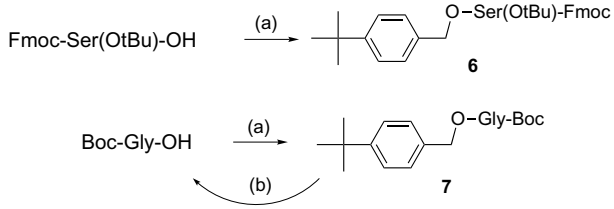
Compounds	Retention time (min) with mobile phase A	Retention time (min) with mobile phase B
Benzyl alcohol	5	5.6
Benzoic acid	15	Broad
4-*tert*-Butylphenol	91	85
tert-Butyl ester **1**	78	87
Acid **2**	70	81
Amine **3**	40	90
Tagged His ester **4**	54	80
Tagged Tyr ester **5**	88	90

Mobile phase A: a linear gradient from 0% methanol to 100% methanol in 0.5% TFA/water over 3 h. Mobile phase B: a linear gradient from 0% methanol to 100% methanol in water over 3 h. Flow rate: 1 mL/min; column size: 25 cm × 4.6 mm.

are very close to that of 4-*tert*-butylphenol and very different from two controlled compounds (benzyl alcohol and benzoic acid) with the exceptions of compounds **3** and **4**, which bear a basic nitrogen. Upon removing TFA from the mobile phase, retention times of compounds **3** and **4** become comparable to that of 4-*tert*-butylphenol, suggesting that protonation of the basic nitrogen in these compounds is responsible for their short retention time with TFA-containing mobile phase. In this cyclodextrin based chromatographic system, the retention mechanism is hydrophobic in nature. Therefore, a highly hydrophilic functional group such as ammonium ion could influence the retention time of these tagged compounds. However, other polar functional groups such as carboxylic acid appear to have little impact on the chromatographic retention of these tagged compounds. For example, the retention times of *tert*-butyl ester **1** and free acid **2** are almost identical. Even for these basic nitrogen-containing compounds **3** and **4**, the retention times are still significantly higher than those of the controlled compounds benzyl alcohol and benzoic acid. Moreover, for the intended tag application, TFA is required only when acidic non-tagged molecules exist, as they tend to tail in the absence of acid in the mobile phase. Linkage of the 4-*tert*-butyphenyl to the target molecule seems to have little impact on the retention behavior, as the linkage through a CH_2 group instead of oxygen in compound **3** does not reduce its retention times when compared to tagged His ester **4**. These results indicate that the 4-*tert*-butylphenyl has the potential to be an efficient and general tag for solution-phase organic synthesis.

A model study was then performed to test the practicality of this tag strategy. For this purpose, 4-(*tert*-butyl)benzyl bromide was chosen as the tagging reagent, as it can be removed cleanly by catalytic hydrogenolysis. A commercially available amino acid derivative, Fmoc-Ser(*t*Bu)-OH, was tagged according to the conditions in Scheme 2. Excess amount (2 equiv) of starting material Fmoc-Ser(*t*Bu)-OH was used in the reaction and the reaction mixture was evaluated directly by HPLC. It was found that with mobile phase up to 50% methanol in 0.5% TFA in H_2O, the tagged amino acid is efficiently retained by the beta-cyclodextrin stationary phase even when injected in large amounts. Subsequently, a protocol involving eluting the column with 40% methanol in 0.5% TFA in H_2O until all impurities were eluted, and then switching to a gradient up to 100% methanol was employed to elute the tagged Fmoc-Ser(*t*Bu)-OH product. An aliquot of reaction mixture that contains roughly 20 mg of reaction product was successfully separated in one run using just the analytical HPLC column mentioned earlier by following this protocol with a recovery yield over 95%. This purification protocol was then streamlined by changing directly to a pure methanol mobile phase from 40% methanol in 0.5%TFA/water to elute the tagged product. By following this streamlined protocol, tagged Gly **7** was also successfully isolated.

Another concern with the tagging system is the ease with which one can remove the tag from the target molecule after separation. In this particular example, the tag can be removed conveniently by catalytic hydrogenolysis. For example, the catalytic hydrogenolysis to recover



Scheme 2. Tag attachment and removal. (a) Cs_2CO_3, 4-(*tert*-butyl)benzyl bromide; (b) H_2, Pd/C.

Boc-glycine from its tagged form **7** was effected in 3 h (Scheme 2). Cleavage was clean and after workup, Boc-glycine was obtained in quantitative yield. The hydrogenation by-product 4-*tert*-butyltoluene (bp 191 °C) was removed by vacuum evaporation.

These experiments demonstrate the potential utility of the 4-*tert*-butylphenyl tagging strategy for solution phase synthesis. Simplicity of this tagging strategy bodes well for the practical application of this method in solution phase synthesis.

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