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Preparation of MBHA resin by benzotriazole-mediated amidoalkylation

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ABSTRACT

MBHA (4-methylbenzhydrylamine) resin is widely used as a solid support for the synthesis of carboxamides or peptide C-terminal amides. Herein, we report a new method for synthesizing MBHA resin by benzotriazole-mediated amidoalkylation. MBHA resin was efficiently prepared with *N*-[(benzotriazol-1-yl)(*p*-tolyl)methyl]formamide or *N*-[formamido(*p*-tolyl)methyl]formamide, and it showed excellent properties as a solid support.

Peptide C-terminal amides are widely employed as peptide drugs, mainly due to their increased biological activity and resistance to biological degradation.[1] Such peptide amides are usually synthesized by solid-phase methods, in which amino acids are coupled to the amino-functionalized polymeric supports, and the desired peptides with the carboxamide group at C-terminal are recovered from the supports after elongation. Since benzhydrylamine (BHA) resins were first introduced for the synthesis of peptide amides, a variety of the amino-functionalized handles or supports have been developed and adjusted for optimal cleavage conditions. Mild acid-sensitive linker systems, such as Rink amide linker,[2] xanthenyl linker,[3] PAL handle[4] and dibenzocyclohepta-1,4-dienylamine linker,[5] have been utilized in solid-phase peptide synthesis (SPPS) using the Fmoc/*t*-Bu strategy. Nonetheless, BHA linkers remain important in the synthesis of peptide amides using the Boc/Bzl strategy due to their simple structures and relatively facile preparation. Among BHA resins, the 4-methyl derivative improved the efficiency of the synthesis of model peptide carboxamides.[6]

Initially, in order to introduce BHA structures onto polystyrene (PS) resin beads, three synthetic methods were employed: benzoyl group reduction followed by bromination and ammonolysis,[7] oxime group reduction,[7] and direct benzoyl group reduction by Leuckart reduction.[8] However, these methods have problems such as crosslinking or formation of secondary amine groups. Furthermore, the Leuckart reduction was too sensitive to the reaction conditions. Alternatively, *N*-(α-chlorobenzyl)phthalimides were used to incorporate BHA groups onto PS resin.[9,10] Despite the facile amino-functionalization, tedious reaction steps were required for their preparation. Recently, BHA structures were also constructed by reducing the imine group obtained from lithiated PS resin and substituted benzonitriles.[11]

On the other hand, α-amidoalkylation has long been adopted to introduce α-aminoalkyl groups into aromatic compounds.[12] *N*-(α-Functionalized alkyl)carboxylic amides or carbamates are usually employed under acidic conditions, where a variety of functional groups, such as hydroxyl, halogen, carboxylate, alkoxide, carboxylic amide and carbamate, can be used as the α-functional group. However, the reaction conditions were too harsh, and strong acid or high temperature was mandatory for significant yield of amidoalkylated products, except for the highly activated aromatic systems.

Meanwhile, Katritzky's group has studied on the benzotriazole-mediated reaction extensively and revealed that benzotriazole could act as a leaving group in the electrophilic aromatic substitution.[13] In particular, benzotriazole-functionalized amides were more readily prepared than other α-functionalized amides, and were successfully employed in the α-amidoalkylation of activated aromatic compounds.[14] However, the α-amidoalkylation of unactivated aromatic compounds has not been described until now. In this Letter, we report the benzotriazole-mediated α-amidoalkylation of unactivated aromatic compounds and its application to the preparation of MBHA resin.

Our new linker system, *N*-[(benzotriazol-1-yl)(*p*-tolyl)methyl]-formamide (**1**), consists of an alkyl group, a carboxylic amide and 1-benzotriazole. Formamide was selected as an amide group because the formyl group can be removed easily after the aromatic substitution reaction. Considering the structure of MBHA resin, the *p*-tolyl group was employed as an alkyl group. Thus, the benzotriazolyl linker (**1**) was prepared from *p*-tolualdehyde, formamide

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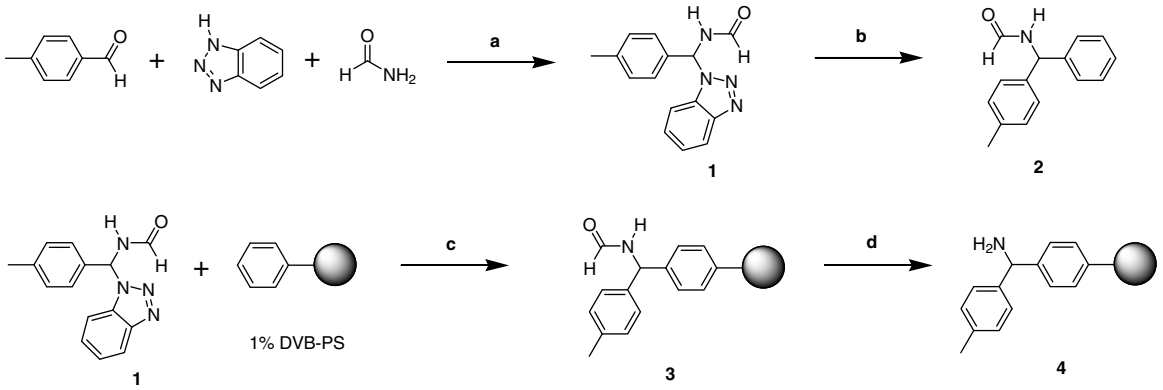


Scheme 1. Synthesis of benzotriazolyl linker (**1**) and MBHA resin (**4**). Reagents and conditions: (a) toluene, reflux, 24 h, 14% yield; (b) $AlCl_3$, benzene, DCE, reflux, 72 h, 12% yield; (c) $AlCl_3$, DCE, reflux, 72 h; (d) 35% HCl, EtOH, reflux, 12 h.

and 1-benzotriazole.[15] The benzotriazolyl linker (**1**) was then reacted with benzene in the presence of aluminium chloride to obtain *N*-[phenyl(*p*-tolyl)methyl]formamide (**2**) (Scheme 1).[16] Despite the long reaction time (72 h) and the low yield (12%), this reaction demonstrated the possibility of α-amidoalkylation of unactivated aromatic compounds such as benzene. Moreover, since ~10% functionalization of phenyl rings in PS resin corresponds to a loading capacity of about 1.0 mmol/g resin and this loading level is sufficiently high for SPPS, the relatively low yield (12%) in the α-amidoalkylation is not considered to pose any problems for the synthesis of MBHA resin.

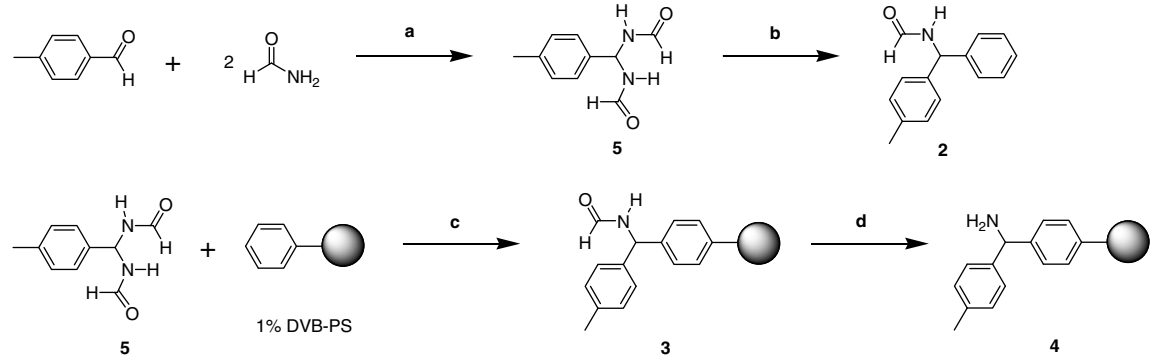
In order to prepare MBHA resin by this method, the benzotriazolyl linker (**1**) was similarly reacted with 1% DVB-crosslinked PS resin to yield [formamido(*p*-tolyl)methyl] PS resin (**3**).[17] The existence of formyl groups in the resin (**3**) was confirmed by FT-IR (amide carbonyl band at 1684 cm^{-1}) and by the nitrogen contents in the elementary analysis (see Supplementary data). MBHA resin (**4**) was obtained by hydrolyzing the resin (**3**), and its loading capacity was determined to be 0.97 mmol/g resin by Fmoc titration after coupling with Fmoc-Gly-OH (Scheme 1).

Although the benzotriazolyl linker (**1**) afforded MBHA resin of high-loading capacity, a more efficient route was required owing to its low yield (14%). *N*,*N*′-Alkylidene bis(amide) is an alternative because it can be obtained in a one-step reaction.[12] For such a linker, *N*-[formamido(*p*-tolyl)methyl]formamide (**5**) was synthesized from *p*-tolualdehyde and formamide in 63% yield (Scheme 2). Bis(formamide) linker (**5**) was easily obtained by filtering the reaction mixture without any further purification steps.

While bis(formamide) linker (**5**) is unreactive in electrophilic aromatic substitution, its reactivity can be improved by adding 1-benzotriazole. In the benzotriazole-catalyzed reaction, 1-benzotriazole can replace one of two formamides in the presence of aluminium chloride and the resulting benzotriazolyl linker (**1**) can be incorporated into the α-amidoalkylation. Accordingly, catalytic amounts of 1-benzotriazole were used in the α-amidoalkylation of benzene with *N*-[formamido(*p*-tolyl)methyl]formamide (**5**), and *N*-[phenyl(*p*-tolyl)methyl]formamide (**2**) was obtained in 13% yield (Scheme 2). Similarly, the benzotriazole-catalyzed amidoalkylation of 1% DVB-PS resin afforded [formamido(*p*-tolyl)-methyl] PS resin (**3**). After hydrolysis of the resin (**3**), high-loading capacity MBHA resin (1.03 mmol/g resin, confirmed by Fmoc-titration after coupling with Fmoc-Gly-OH) was prepared (Scheme 2).

MBHA resins obtained by benzotriazole-mediated reactions, such as the benzotriazolyl linker method and the benzotriazole-catalyzed method, not only possess sufficiently high-loading levels for SPPS, but also exhibit clean surface morphology and good swelling properties (see Supplementary data). On the other hand, in order to evaluate the performance of the MBHA resin in peptide synthesis, a model peptide, Leu-enkephalin (YGGFL-NH_2), was synthesized on Fmoc-Leu-NH-MBHA resin (0.47 mmol/g resin) using Boc/Bzl chemistry after removing the Fmoc-group with 20% piperidine in NMP. After cleavage with TFMSA, the crude product was analyzed by HPLC. In the HPLC chromatogram, one peak (88% purity) was detected, which was found to be Leu-enkephalin by MALDI-TOF (Fig. 1).

In summary, benzotriazole-mediated amidoalkylation was accomplished on the unactivated aromatic compounds. Benzotriazolyl linker (**1**), or bis(formamide) linker (**5**) combined with catalytic benzotriazole, was reacted with benzene to give the formamidoalkylated product. Using this method, MBHA resin was successfully synthesized after reaction with 1% DVB-PS resin, and



Scheme 2. Synthesis of bis(formamide) linker (**5**) and MBHA resin (**4**). Reagents and conditions: (a) cat. H_2SO_4, toluene, reflux, 18 h, 63% yield; (b) 1-benzotriazole (0.2 equiv), $AlCl_3$, benzene, DCE, reflux, 72 h, 13% yield; (c) 1-benzotriazole (0.2 equiv), $AlCl_3$, DCE, reflux, 72 h; (d) 35% HCl, EtOH, reflux, 12 h.

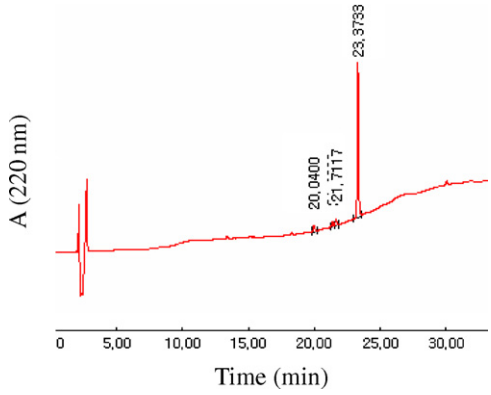


Figure 1. HPLC chromatogram of crude YGGFL-NH_2 synthesized on MBHA resin (MALDI-TOF: calcd 577.3 for YGGFL-NH_2 $[M+Na]^+$, found 577.3).

it had good properties as a solid support for SPPS. Considering the facile availability of bis(formamide) linker (**5**), the benzotriazole-catalyzed version is believed to be a simple, beneficial method for the preparation of MBHA resin.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.06.110.

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16. Preparation of (**2**). To a suspension of the benzotriazolyl linker (**1**) (1.0 g, 0.8 equiv) in benzene (25 mL) and DCE (25 mL) was added aluminium chloride (1.0 g, 1.6 equiv). The reaction mixture was heated to reflux for 72 h under nitrogen atmosphere. The reaction mixture was quenched by adding 3 N HCl (20 mL) after cooling to 0 °C, and transferred to a separatory funnel. The organic phase was separated, and washed with 3 N HCl (30 mL × 2) and H_2O (30 mL). The organic layer was dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude product was purified by column chromatography using *n*-Hex/EtOAc (2:1) as eluent (R_f = 0.17), *N*-[phenyl(*p*-tolyl)methyl]formamide (**2**) was obtained as white solid (0.10 g, 12% yield).
17. Preparation of (**3**). To a suspension of PS resin (5.0 g, 1.0 equiv, 1% DVB-crosslinked, 100–200 mesh) in DCE (80 mL) were added benzotriazolyl linker (**1**) (7.7 g, 0.8 equiv) and aluminium chloride (7.7 g, 1.6 equiv). The reaction mixture was heated to reflux for 72 h under nitrogen atmosphere. The reaction mixture was quenched by adding 3 N HCl (20 mL)/THF (10 mL) after cooling to 0 °C, and the resin was collected by filtration. The resin was washed with THF (75 mL)/3 N HCl (25 mL) (×2), THF (75 mL)/water (25 mL) (×2), THF (100 mL × 2), DCM (100 mL × 2) and MeOH (100 mL × 2), and dried in vacuo to yield [formamido(*p*-tolyl)methyl] PS resin (**3**). FT-IR: amide carbonyl band at 1684 cm^{-1}, N analysis: 1.695%, the loading level 1.21 mmol/g resin.