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An efficient approach to prepare glyoxylyl functionality on solid-support

Qingchai Xu and Kit S. Lam*

UC Davis Cancer Center, Division of Hematology & Oncology, Department of Internal Medicine, University of California Davis, 4501 X Street, Sacramento, CA 95817, USA

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Abstract—Glyoxylyl group on solid-support is an attractive intermediate for solid-phase synthesis, and combinatorial synthesis. We report here a novel method to generate glyoxylyl functionality on solid-support that involves coupling of acrylic acid, followed by oxidation of the double bond. This highly efficient and versatile approach is compatible with hydroxyl-resins, amino-resins and other solid supports such as glass surfaces. © 2002 Elsevier Science Ltd. All rights reserved.

Aldehyde functionalities are useful intermediates for many different types of reactions, including multi-component reactions such as Ugi[1] and Mannich[2] reactions. The glyoxylyl group, a special form of aldehyde, has been used in chemoselective ligations of unprotected peptides or peptide-proteins in aqueous medium.[3,4] In these ligation methods, glyoxyl aldehyde reacts with unprotonated weak bases including amino-oxy, 1,2-aminothiol and hydrazides under mild acidic conditions, but it does not react with relatively stronger basic groups such as $N^{\alpha-}$ and the side-chain amino groups of peptides or proteins. Recently, resin bound glyoxylyl aldehydes have been used for solid-phase organic synthesis and combinatorial chemistry.[5]

A glyoxylyl group can be easily generated in solution via periodate oxidation of a terminal serine or threonine residue. In contrast, preparation of a resin-bound glyoxylyl group, via oxidation of on-resin terminal serine or threonine, requires orthogonal protection of the amino acids, and the yield is usually low.[5,6] Better results have recently been achieved with methods involving periodate oxidation of diols, but these methods are only compatible with particular resins due to problems associated with immobilization of the diols.[5,7] Therefore, an efficient and general approach for the preparation of a glyoxylyl group is needed.

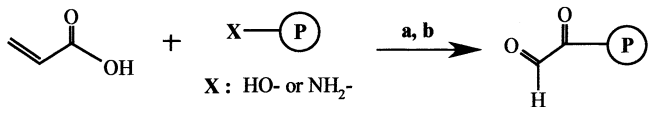

Figure 1. Preparation of glyoxylyl resin. (a) 0.5 equiv. DIC in DMF, (b) 10% $NaIO_4$, catalytic amount of OsO_4 in H_2O/Diox (1:3), overnight.

We reported on the use of a glass-bound glyoxylyl group to chemoselectively ligate peptides and small molecules to form a chemical microarray, which can then be used as a high-throughput method to analyze different biological interactions.[8] Low yields are associated with glyoxylyl groups generated from (i) periodate oxidation of glass-bound serine or threonine, or (ii) direct conjugation to glass with protected glyoxylic acid. Therefore, more efficient strategies were compared: (i) coupling of acrylic acid onto a solid-support followed by oxidation to an aldehyde, (ii) use of a protected glyoxylic acid for coupling, followed by hydrolysis and (iii) direct coupling of an unprotected glyoxylic acid.

In the first method, acrylic acid was coupled onto an amino or hydroxyl resin via DIC-mediated symmetric anhydride activation, followed by OsO_4-catalyzed oxidation with sodium periodate (Fig. 1). Wang resin (hydroxyl-resin) and Phe-Wang resin (amino-resin) were used as the solid-support. Subsequent oxidation was accomplished by adding a mixture of sodium periodate solution containing a catalytic amount of OsO_4 in H_2O/dioxane.

Keywords: glyoxylyl functionality; solid-phase; glass slide; aldehydes; chemoselective ligation.

* Corresponding author. Present address: UC Davis Cancer Center, 4501 X Street, Sacramento, CA 95817, USA. Tel.: 916-734-8012; fax: 916-734-7946; e-mail: kit.lam@ucdmc.ucdavis.edu

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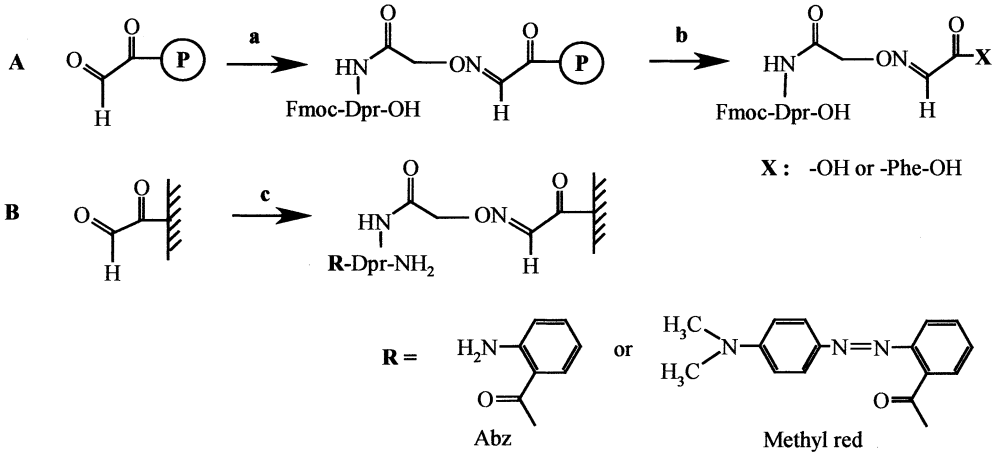

Figure 2. Detection of solid supported glyoxylyl group. **A** on Wang resin; **B** on glass slides. (a) Ligation with Fmoc-Dpr(Aoa)-OH (1 mg/mL) in DMSO/H_2O (1:1, pH 5.0), overnight; (b) 50% TFA/H_2O (1:1), 1 h; (c) ligation with Abz-Dpr(Aoa)-NH_2 or methyl red-Dpr(Aoa)-NH_2 (1 mg/mL) in DMSO/H_2O (1:1, pH 5.0), overnight.

To determine the efficiency of this approach, Fmoc-Dpr(Aoa)-OH (N^{α}-Fmoc-N^{β}-(amino-oxyacetyl)-L-diaminopropionic acid) was coupled to the glyoxylyl resin via an oxime bond (Fig. 2A). The ligation reaction was performed in H_2O/DMSO (pH 5.0, 1:1) overnight. The oxime product was released from the Wang resin or the Phe-Wang resin with 50% TFA/DCM. There was only one major peak evident by chromatography (C-18 reverse-phase HPLC) from the Wang resin and Phe-Wang resin releasates, which suggested that they were over 90% pure. ES-MS confirmed that the chemical identities of these peaks were in fact the expected oxime products. The absence of acrylic acylated products on the HPLC chromatogram indicated that the oxidation reaction was complete. In another experiment using the same conditions, we successfully ligated a synthetic unprotected peptide (Leu-His-Pro-Gln-Phe-Dpr(Aoa)-NH_2) containing an amino-oxy group to the glyoxylyl-functionalized Wang resin.[9]

Encouraged by these results, we then used this method to prepare glyoxylyl-functionalized glass slides. The glass slides were first treated with 3-aminopropyl triethoxysilane yielding amino-glass slides.[8] Acrylic acid was then coupled onto the amino-modified glass slides. The modification to the glass-slide surfaces before and after acylation was confirmed using 1% aqueous bromophenol blue solution to test for the presence or absence of the amine groups on glass surfaces. Subsequent periodate oxidation was carried out in aqueous media. The slides were then washed with water and dried under nitrogen. In order to prove the presence of a glyoxylyl group on the glass surface, a proper detection method had to be developed. To accomplish this, we prepared a fluorescent compound (Abz-Dpr(Aoa)-NH_2, Abz: abbreviation for 2-aminobenzoic acid) and a dye derivative (methyl red-Dpr(Aoa)-NH_2); both contain an amino-oxy moiety (Aoa) for oxime ligation onto the modified glass surface (Fig. 2B).[10] Both compounds were dissolved in DMSO/water (pH 5.0, 1:1 mix) at a final concentration of 1 mg/mL. Five microliters of each of the indicator solutions were manually spotted onto the glass slides, and the spotted slides were incubated overnight at room temperature in a humidified container. After thorough washing with H_2O, DMSO and MeOH, the slides spotted with Abz-Dpr(Aoa)-NH_2 were examined under a fluorescent microscope. Fluorescent (bright blue) spots were clearly visible indicating that the ligation of the Abz-compound was successful. Similarly, light yellow spots were seen on the glass slides spotted with methyl red-Dpr(Aoa)-NH_2.[11]

For the second method, we investigated the use of dimethyl acetal protected glyoxylic acid (dimethoxyacetic acid) as the derivatizing agent (Fig. 3). Dimethyoxyacetic acid was prepared by hydrolysis of commercially available ethyldimethoxyacetate with aqueous NaOH.[12] DIC-mediated coupling of the acid to either the Phe-Wang resin or amino-slides was performed in 5% DIEA in DMF. Dimethoxyacetylated Phe was obtained in good purity after TFA-cleavage from the Wang resin. This compound was stable to both TFA and diluted HCl but was rapidly hydrolyzed in 12N HCl. The glyoxylyl slides were generated after treatment of the dimethoxyacetylated glass slides with 1 N HCl for 1 h at 0–5°C.

We also tried to use unprotected glyoxylic acid monohydrate to directly derivatize the Wang resin via DIC coupling. Fmoc-Dpr(Aoa)-OH was ligated to this derivatized resin, and the TFA-cleavage products yielded the expected compound with greater than 80% purity using C-18 reverse-phase HPLC. However, attempts to modify an amino resin (Phe-Wang resin) with unprotected glyoxylic acid were not successful.

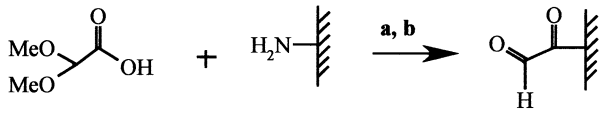

Figure 3. Preparation of glass-slide supported glyoxylic aldehyde via dimethoxyacetic acid. (a) DIC, in 5% DIEA/DMF; (b) 12N HCl, 1 h at 0–5°C.

In summary, three different approaches for the preparation of functional glyoxylyl groups onto solid supports have been investigated. Our results show that the two-step acrylic acid method is the most efficient and versatile. This approach is compatible with hydroxyl-resins, amino-resins and glass slides. Because the reaction condition is mild, we believe it may also be used to functionalize biomolecules for subsequent bioconjugation. The dimethoxyacetic acid-method requires 12N HCl to liberate the aldehyde group, therefore it will not work in proteins or other biological materials. However, this method is still useful for generating glyoxylyl functionality on non-cleavable linkers and acid resistant solid-supports, such as glass slides and TentaGel resins.

Acknowledgements

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9. The peptide Leu-His-Pro-Gln-Phe-Dpr(Aoa)-NH_2 (MW 798.4, purity >95% after purification) used for ligation was synthesized on a Rink-Amide MBHA resin employing the standard Fmoc-SPPS procedure. After ligation the immobilized peptide was cleaved by 50% TFA/DCM and subjected to analytical RP-HPLC. On the HPLC chromatogram a major peak at 7.6 min (>85%) was observed. ES-MS analysis of the major component gave one major mass signal at 877.6 $[M+Na]^+$, which corresponds with the expected compound, Leu-His-Pro-Gln-Phe-Dpr(CO-CH_2ON=CHCOOH)-NH_2.
10. Abz-Dpr(Aoa)-NH_2 and methyl red-Dpr(Aoa)-NH_2 were prepared by coupling of Boc-2-Abz and Methyl red, respectively, onto Dpr(Boc-Aoa)-Rink Amide resin, followed by TFA cleavage.
11. Full details will be published along with chemical microarray study on due time.
12. To a pre-cooled solution of methyldimethoxyacetate (1 mmol) in methanol (1 mL), 1.1 mL 1.0 N NaOH was added. The mixture was stirred at rt for 3 h, concentrated under vacuum and the pH of the final concentrate was carefully adjusted to 5.0 with 0.1N HCl. The final mixture was frozen, and lyophilized to dryness.