Simple and Practical Real-Time Analysis of Solid-Phase Reactions by Thin-Layer Chromatography

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Abstract
Solid-phase synthesis is a practical approach for simplifying the time-consuming and routine purification steps in the preparation of numerous naturally occurring molecules; however, studying such reactions is difficult due to the lack of a convenient monitoring method. By using thin-layer chromatography in conjunction with a photolabile linker on a resin, we developed a convenient and simple method for monitoring solid-phase reactions in real time by thin-layer chromatography. This method provides a user-friendly protocol for examining reaction conditions for solid-state syntheses.

Key words solid-phase reactions, real-time analysis, thin-layer chromatography, photolabile linkers, glycosylation, click chemistry

Peptides,1 nucleotides,2 and carbohydrates3 are structurally complex biomolecules that perform a host of crucial biological functions in nature, but the short supply of some of these biomolecules from natural sources has hampered their fundamental study. Consequently, in the last few decades, numerous methods have been developed for producing tailored building blocks for manipulating biologically important peptides, nucleotides, and saccharides (for example, Scheme 1a).4 The chemical synthesis of structurally complex molecules generally requires multiple synthetic steps and routine purifications. However, since the development by Merrifield of a novel technique for peptide synthesis (Scheme 1b),5,6 the number of preparations of complex biomolecules has increased as a result of the use of solid-phase synthesis methods. Solid-phase synthesis facilitates the purification process by permitting simple washing with appropriate solvents and filtrations of functional resins bearing the targeted molecules. This procedure is key to overcoming the challenge of synthesizing complex molecules.7 For example, Seeberger achieved an automated synthesis of a 30-mer mannoside by using a modified Merrifield resin; this protocol provides a valuable method for solid-phase assembly of polysaccharides (Scheme 1c).6

Currently, a method commonly used to study target-oriented solid-phase syntheses involves cleaving the resin in the final stage after multiple synthetic steps. Numerous modern analytical methods involving mass spectroscopy (MS),7 IR spectroscopy,8 and NMR spectroscopy9 have been developed and used to examine targeted molecules in the late stages of their syntheses. However, solid-phase syntheses require excess amounts of reagents and relatively long reaction times, so that real-time analyses of reaction conditions for individual synthetic steps remain challenging.

Because thin-layer chromatography (TLC) has been used to separate and analyze natural products since the 1960s,10 it has become a common and indispensable technique for studying chemical transformations in chemical synthesis. Because nitrobenzyl ether-based Merrifield resin has been demonstrated to be photocleavable under UV irradiation at a wavelength of 254 nm,6,11 we surmised that a combination of a TLC technique with the use of a capillary might be suitable for examining stepwise transformations. A capillary enables the carrying and maintenance of beads in a solution through its capillarity, where beads swell after the excess reactants are washed out with CH2Cl2 and methanol. Additional exposure to UV radiation then induces photocleavage of the resin within the capillary, and spotting of the resulting mixture onto a TLC plate permits the study of solid-phase reactions in real time (Scheme 2).
We began our model analysis with an examination of a TolSCl/AgOTf-mediated glycosylation\(^\text{12}\) of resin 1 with the 4,6-O-benzylidenegalactoside derivative 2a to give the galactoside 3a (Scheme 3).\(^\text{13}\) When the reaction mixture had been shaken at room temperature for three hours, a capillary was used to extract some resin particles (approximate-ly 20 beads) from the reaction mixture for analysis. The beads were washed three times each with CH\(_2\)Cl\(_2\) and methanol to remove excess reagents, and then the capillary containing the resin beads 3a and CH\(_2\)Cl\(_2\) was irradiated with a UV lamp (254 nm) for 10–15 minutes. The reaction mixture in the capillary was then spotted onto a TLC plate\(^\text{14}\)
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(see Scheme 2; see also Supporting Information, Figures S1 and S2). Staining with Hanessian's reagent (ceric ammonium molybdate) and subsequent TLC revealed the presence of galactoside adduct 4a as a single anomer, together with unreacted linker 5 [Scheme 3(a)]; these results could be compared with those of the corresponding solution-phase reaction [Scheme 3(b)]. Photoinduced cleavage of the Merrifield resin 3a under UV irradiation (254 nm) for one hour gave galactoside 4a in 56% isolated yield ($\beta/\alpha > 20:1$), together with a 40% isolated yield of the unreacted linker 5. This encouraging outcome showed that this is a user-friendly protocol for the real-time study of solid-phase glycosylations.

We then investigated a question pertaining to the photoinduced conversion of resin 1 into linker 5. $^1$H NMR monitoring showed that on exposure to UV irradiation (254 nm) for 5, 10, or 15 minutes resin 1 was converted into linker 5 in 12, 37, and 52% yield, respectively. Further irradiation of resin 1 for 20, 30, or 40 minutes gave linker 5 in 82, 79 and 81% yield, respectively (see Supporting Information, Figure S3). However, decomposition of resin 1 was observed at longer UV irradiation times (>120 minutes).15

Next, we explored the substrate scope in glycosylations by using our real-time analysis protocol (Table 1). NIS/TfOH conditions were chosen for these solid-phase glycosylations because of the insolubility of AgOTf. A NIS/TfOH-mediated reaction of linker 1 with fully benzylated thiogalactoside 2b resulted in the adduct 4b. TLC analysis indicated the presence of clean glycosylation product 4b in 90% isolated yield ($\beta/\alpha > 20:1$), with a complete conversion of linker 5. Additionally, linker 5 was not observed after the photoinduced cleavage (Table 1, entry 1). Similarly, solid-phase glycosylation of linker 1 with sugar 2c gave the aminopentyl glucoside 4c in a 31% isolated yield ($\beta/\alpha > 20:1$), together with 5 in a 35% isolated yield (entry 2); similar treatment of sugar 2d gave no 4d and an 85% isolated yield of 5 (entry 3).16

Next, we examined the esterification of amino acids with resin 1. Tyrosine derivative 6a, serine derivative 6b, and threonine derivative 6c were coupled with resin 1 through a DIC/HOBt-mediated esterification process, followed by the standard cleavage of the resin, to give esters 7a, 7b, and 7c, respectively, in two-step yields of 60, 93, and 82% (entries 4–6). Our analytical protocol permitted accurate real-time analyses.17

Scheme 3 Model analysis of a galactoside in (a) solid-phase and (b) solution-phase glycosylation, together with the corresponding real-time TLC analyses. The standard lane was spotted with compound 4a or 5 as a standard, the reaction lane was spotted with the reaction mixture, and the co-pot lane was spotted with both the standard and the reaction mixture.
We also explored a click reaction by using our real-time TLC method. A direct O-propargylation of resin 1 was performed by a NaH-mediated reaction in DMF solution to give alkyne 8. Additionally, a Cu nanoparticle/cluster-catalyzed click reaction of alkyne 8 with azido compound 9 gave the desired adduct 10; in this reaction, the Cu nanoparticle/cluster was formed in situ through reduction of Cu(II) with hydrazine. Both intermediates 8 and 9 were observable on the TLC plates. The final photoinduced cleavage of the resin produced triazole 11 in 43% overall yield over three steps (Scheme 4). Our simple analytical procedure provided accurate results regarding the reaction transformation; consequently, extra reagents and repeated steps were not needed.
We finally determined the applicability of our real-time TLC examination protocol to the esterification of resin 1 with amino acids, followed by glycosylation. A microwave-assisted DIC/HOBt-mediated esterification of resin 1 with amino acid 6c or 6d gave the amino esters 12a and 12b, respectively. Subsequent desilylation of 12a and 12b with a large excess of TBAF gave alcohols 13a and 13b, respectively. Next, glycosylations of donor 2c with alcohols 13a and 13b were performed under TolSCl/AgOTf, NIS/TfOH, and BSP/Tf2O conditions. However, only the BSP/Tf2O conditions gave the desired glucose adducts 14a and 14b. In the final photoinduced cleavage of the resin, the targeted adducts 15a and 15b were obtained in 50% (β/α = 1:1) and 66% (β/α > 20:1) overall yield, respectively, over the four steps. The proposed TLC method permitted real-time stepwise examinations of the preparation of 15a and 15b from resin 1 (Scheme 5).20

A simple and user-friendly protocol for the real-time study of solid-phase reactions was developed by using the photocleavable Merrifield resin 1 and TLC. In particular, stepwise transformations such as glycosylation, peptide esterification, and a Cu nanoparticle/cluster-catalyzed click reaction were examined by using the proposed protocol, and the results showed a high degree of accuracy. We suggest that this protocol provides a practical method for increasing the rate of identification of appropriate conditions for sequential reactions in solid-phase syntheses in chemical laboratories.

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Supporting Information
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References and Notes

Scheme 5 Sequential esterification, deprotection, and glycosylation by solid-phase synthesis. Eluting solvent: hexane–EtOAc (1:1).


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(13) **Solid-Phase Glycosylation Procedure for the Synthesis of Glycoside 4a**

Resin 1 (500 mg, 0.1 mmol/g) was swollen in CH2Cl2 (4 mL) for 2 h. Swollen 1 in CH2Cl2 was mixed with sugar 2a (139 mg, 0.3 mmol) and TsCl (38 μL, 0.3 mmol), then AgOTf (64 mg, 0.3 mmol) was added and the mixture was kept at r.t. for 3 h. Unreacted reagents were removed by sequential washing with CH2Cl2 and MeOH (×3). The resins in CH2Cl2 were exposed to UV radiation in 1 h, then filtered. The products in the filtrate were purified by flash column chromatography [silica gel, hexane–EtOAc (3:1 for 4a; 1:2 for 5a)] to give 4a as a white solid; yield: 19 mg (56%). Linker 5 was also obtained as a white solid; yield: 5 mg (40%).

(14) **Real-Time Analyses of Solid-Phase Reactions by TLC; General Procedure**

A minute sample of the reaction mixture in the reaction vessel was captured by capillary attraction in a capillary tube (see Supporting Information, Figure S1). The liquid solution in the capillary was absorbed by using a TLC plate, while the resin beads were retained in the capillary (Figure S2). The beads were then washed sequentially with CH2Cl2 and MeOH three times to remove excess reactants. Both CH2Cl2 and MeOH were able to flow into the capillary through capillary attraction, and could be subsequently removed by absorption onto the TLC plate. After the repeated washing steps, the capillary loaded with the appropriate sugar derivate and CH2Cl2 was irradiated with UV radiation for 10–15 minutes, and the resulting reaction mixture from the capillary was spotted onto another TLC plate. After eluting the sample, the TLC plate was stained with Hanessian’s reagent and heated on a hotplate.

(15) **Determination of the Reaction Time for Photocleavage**

Resin 1 (500 mg, 0.1 mmol/g) was immersed in CH2Cl2 (7.8 mL) for 1 h. Nine 0.1 mL aliquots were extracted from the resin solution and exposed to UV radiation (254 nm) for various times (5, 10, 15, 20, 30, 40, 60, 90, or 120 min). Linker 5, obtained after irradiation and the removal of CH2Cl2, was dissolved in CD2Cl2 (0.4 mL) containing (5 × 10^-3)% TMS (v/v) as an internal standard. The results are given in the Supporting Information (Figure S3).

(16) **Solid-phase Glycosylation to Give Products 4b–d; General Procedure**

Swollen resin 1 (500 mg, 0.1 mmol/g) in CH2Cl2 was mixed with the appropriate sugar derivative 2b–d (0.3 mmol) and NIS (56 mg, 0.3 mmol), TFOX (22 μL, 0.3 mmol) was added at -40 °C, and the mixture was maintained at -40 °C for 2–3 h. Unreacted reagents were washed out three times with CH2Cl2 and MeOH. The resins in CH2Cl2 were exposed to UV radiation for 1 h then filtered. The products in the filtrate were purified by flash column chromatography [silica gel, hexane–EtOAc (3:1 for 4b; 4:1 for 4c; 1:2 for 5)].

(17) **Solid-Phase Syntheses of Aminopropyl Esters 7a–c**

A solution of the appropriate amino acid 6a–c (0.5 mmol) and HOBT (68 mg, 0.5 mmol) in DMF (200 μL) was injected into CH2Cl2 (4 mL) containing swollen resin 1 (500 mg, 0.1 mmol/g). A solution of DIC (63 mg, 0.5 mmol) in DMF (200 μL) was dropped into the reaction mixture, followed by the addition of a catalytic amount of DMAP. The microwave-assisted reactions were conducted at 90 °C for 20 min. Unreacted reagents were washed out five times with CH2Cl2 and MeOH. The resin in CH2Cl2 was exposed to UV radiation for 1 h, then filtered. The products in the filtrate were purified by flash column chromatography [silica gel, hexane–EtOAc (2:1 for 7a; 3:1 for 7b; 4:1 for 7c)].

(18) **Triazole 11 by Solid-Phase Click Reaction**

A mixture of resin 1 (500 mg, 0.1 mmol/g) swollen in DMF (2 mL) was slowly added to a 60% dispersion of NaH (50 mg, 0.6 mmol) in mineral oil at 0 °C, and the mixture was stirred at 0 °C for 6 h. Proprargyl bromide (54 μL, 0.56 mmol) was then slowly added at 0 °C, and the mixture was kept at r.t. for 6 h. Unreacted reagents were washed out five times with 1:1 v/v MeOH–H2O and CH2Cl2. The resulting resin 8 was swollen in CH2Cl2 for 2 h. A mixture of resin 8 in 2:2:1 CH2Cl2–MeCN–H2O (2 mL) was treated with azide 9 (79 μL, 0.66 mmol) and CuSO4·5H2O (154 mg, 0.66 mmol), and the mixture was kept at r.t. for 3 h. N2H4·H2O (30 μL, 0.6 mmol) was added, and the mixture was allowed to react for 4 h. Unreacted reagents were washed out five times with CH2Cl2 and MeOH. A mixture of the resin and CH2Cl2 was exposed to a UV lamp for 1 h then filtered. The filtrate was purified by flash column chromatography [silica gel, hexane–EtOAc (5:1)] to give 11 as a brown solid; yield: 9 mg (43% over three steps). Eluting solvent for TLC: hexane–EtOAc (2:1).

(19) **Products 15a and 15b by Sequential Solid-Phase Reactions**

A 1 M soln of TBAF in THF (500 μL, 0.55 mmol) was added drop-wise to a solution of swollen resin 12 (500 mg, 0.1 mmol/g) in CH2Cl2, and the mixture was stirred at r.t. for 4 h. Unreacted reagents were washed out with CH2Cl2 and MeOH to give resin 13. Resin 13 and a solution of glucopyranoside 2c (323 mg, 0.55 mmol) in CH2Cl2 were mixed with 1-[(phenylsulfonyl)piperidide (105 mg, 0.55 mmol) and Ti(O2 (82 μL, 0.55 mmol), and the mixture was stirred at -78 °C for 2–3 h. Unreacted reagents were washed out with CH2Cl2 and MeOH to give resin 14, which was exposed to a UV lamp for 1 h. The filtrate was purified by flash column chromatography [silica gel, hexane–EtOAc (7:1 for 15a, 6:1 for 15b)]. 15a was obtained as a colorless oil; yield 25 mg (50% overall). 15b was also obtained as a colorless oil; yield 33 mg (66%). Eluting solvent for TLC: hexane–EtOAc (1:1).