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

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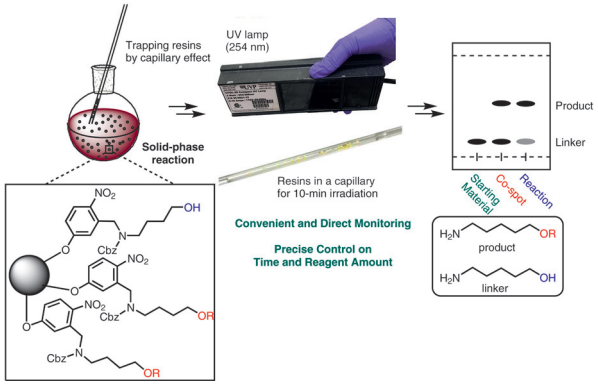

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Abstract Solid-phase synthesis is a practical approach for simplifying the time-consuming and routine purification steps in the preparation of numerous naturally occurring molecules; however, studying such reactions is difficult due to the lack of 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 carbohydrates[3] 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.[5] 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 spectroscopy[9] 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 CH_2Cl_2 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).

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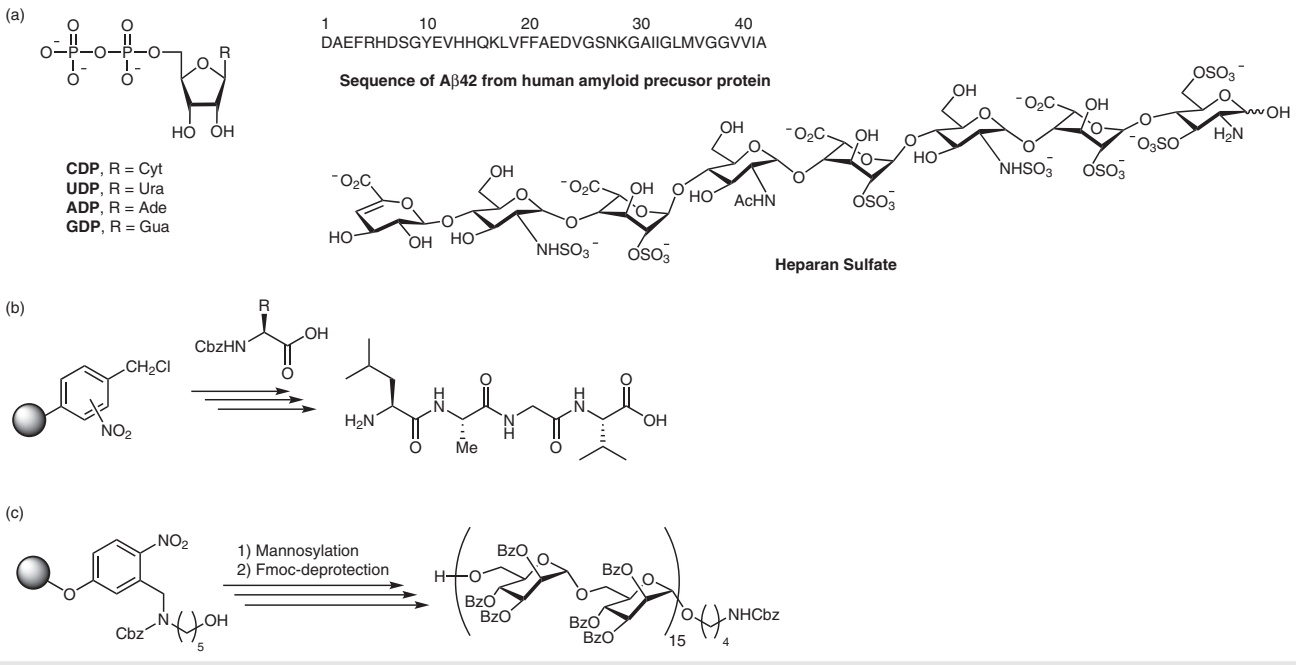
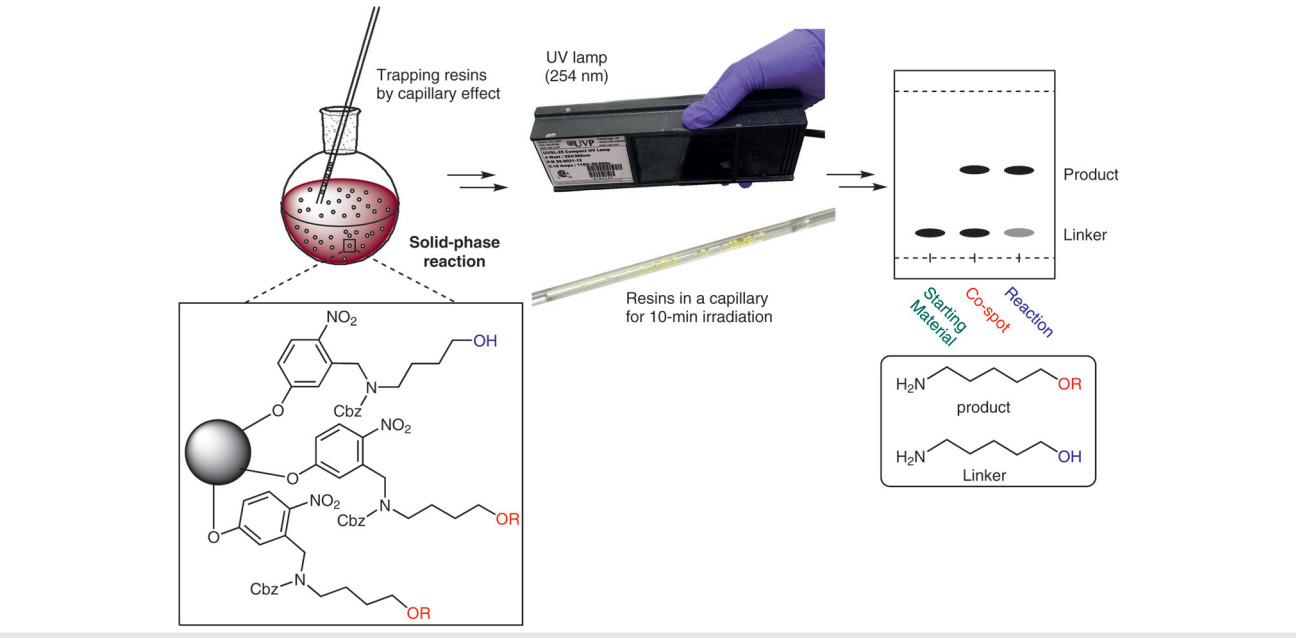


Scheme 1 (a) Representative biomolecules. Solid-phase synthesis of (b) a peptide and (c) a polymannoside.



Scheme 2 General procedure for the real-time analysis of solid-phase reactions by TLC

We began our model analysis with an examination of a TolSCl/AgOTf-mediated glycosylation[12] of resin **1** with the 4,6-*O*-benzylidenegalactoside derivative **2a** to give the galactoside **3a** (Scheme 3).[13] When the reaction mixture had been shaken at room temperature for three hours, a capillary was used to extract some resin particles (approximately 20 beads) from the reaction mixture for analysis. The beads were washed three times each with CH_2Cl_2 and methanol to remove excess reagents, and then the capillary containing the resin beads **3a** and CH_2Cl_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[14]

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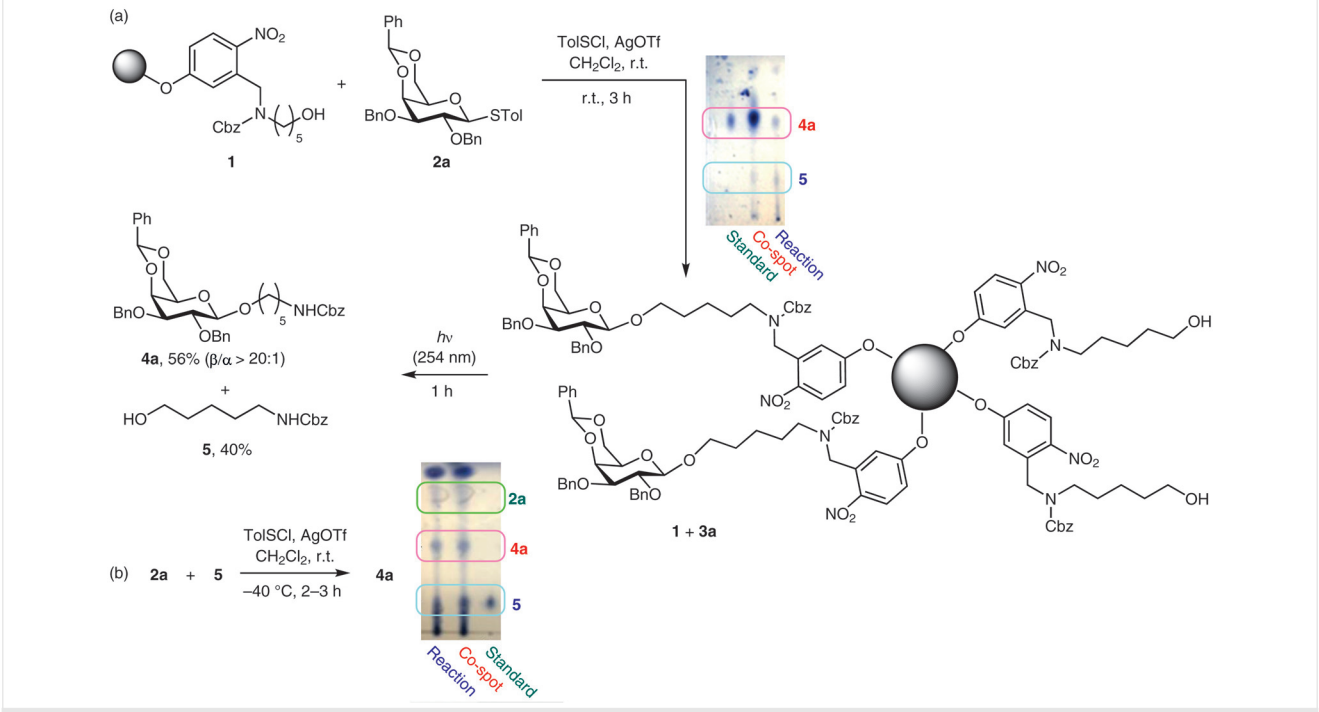
(Scheme 2; see also Supporting Information, Figures S1 and S2). Staining with Hanessian's reagent (ceric ammonium molybdate) and subsequent TLC revealed the presence of galactoside adduct **4a** as a single anomer, together with unreacted linker **5** [Scheme 3(a)]; these results could be compared with 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 (β/α > 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 (β/α > 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 (β/α > 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.

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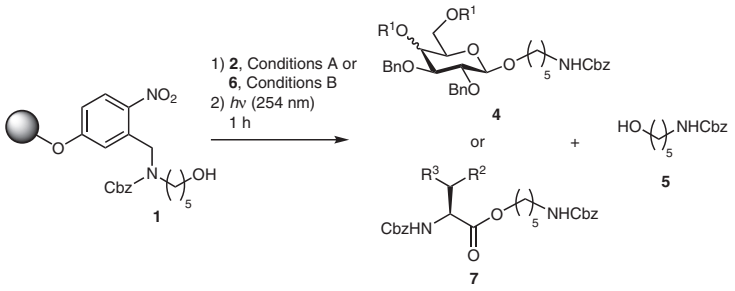
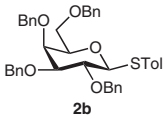
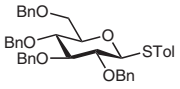
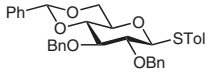
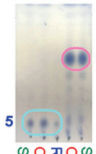
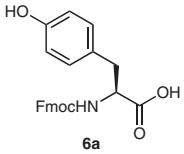
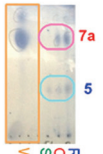
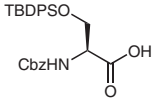
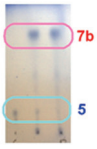
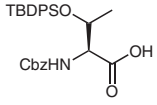
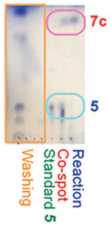
Table 1 Comparison of TLC Results and Actual Yields



Entry	Substrate	Yields	TLC
1[a]	 **2b**	**4b**: 90%(β/α > 20:1) **5**: n.d.[c]	
2[a]	 **2c**	**4c**: 31%(β/α > 20:1) **5**: 38%	
3[a]	 **2d**	**4d**: n.d. **5:** 85%	
4[b]	 **6a**	**7a**: 60% **5**: 25%	
5[b]	 **6b**	**7b**: 93% **5**: n.d.	
6[b]	 **6c**	**7c**: 82% **5**: 14%	

[a] Conditions A: NIS, TfOH, CH_2Cl_2, −40 °C, 2–3 h. Eluting solvent: hexane–EtOAc (1:1 for **4b**; 2:1 for **4c** and **4d**).
[b] Conditions B: DIC, DMAP, HOBt, DMF–CH_2Cl_2 (1:10), MW (30 W, 90 °C, 20 min). Eluting solvent: hexane–EtOAc (1:2 for **7a**; 1:1 for **7b** and **7c**).
[c] n.d. = not detected.

We also explored a click reaction by using our real-time TLC method.[18] 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.[18] 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).[19] Our simple analytical procedure provided accurate results regarding the reaction transformation; consequently, extra reagents and repeated steps were not needed.

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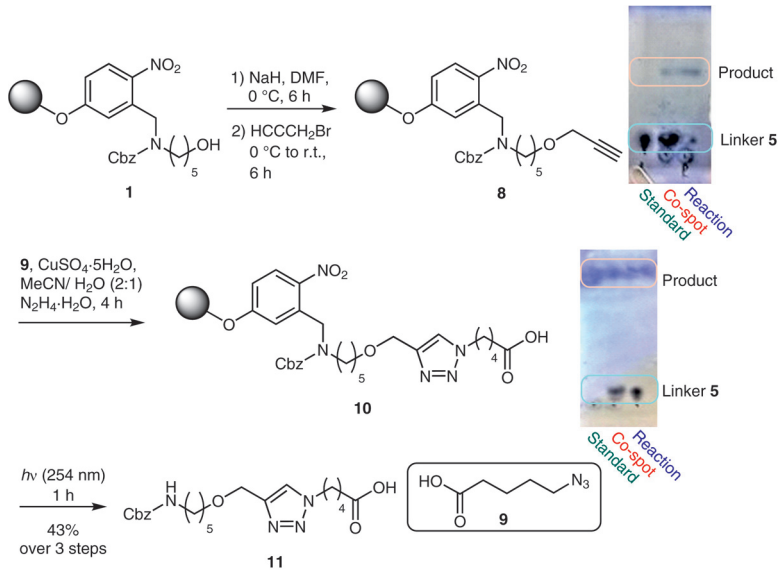

Scheme 4 A click reaction on a resin. Eluting solvent: hexane–EtOAc (2:1).

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/Tf_2O conditions. However, only the BSP/Tf_2O 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

Supporting information for this article is available online at https://doi.org/10.1055/s-0036-1592008.

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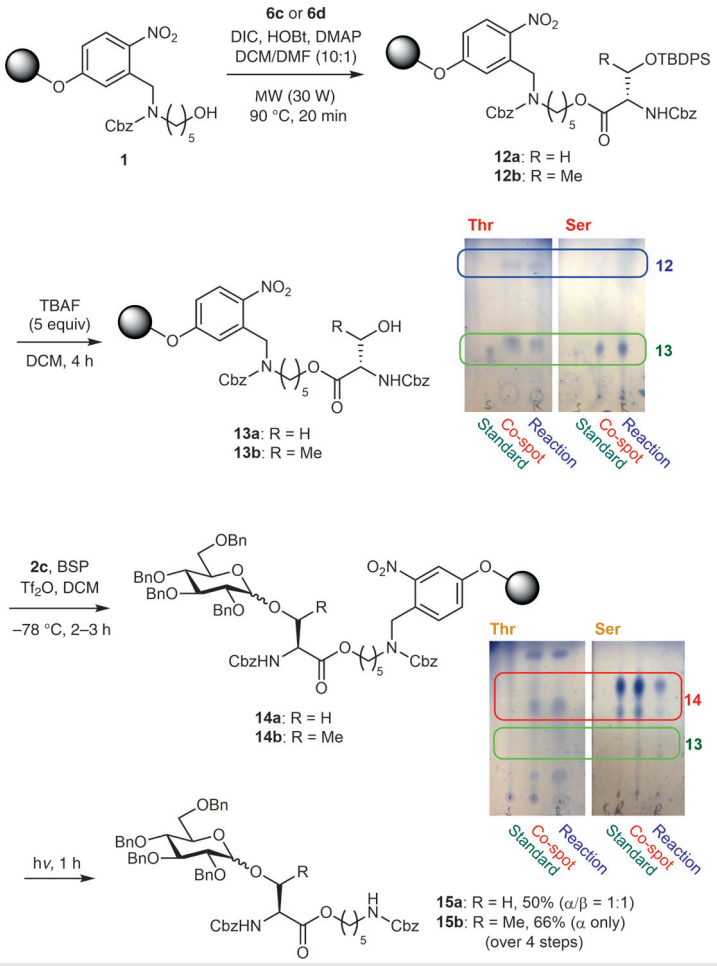

Scheme 5 Sequential esterification, deprotection, and glycosylation by solid-phase synthesis. Eluting solvent: hexane–EtOAc (1:1).

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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 CH_2Cl_2 (4 mL) for 2 h. Swollen **1** in CH_2Cl_2 was mixed with sugar **2a** (139 mg, 0.3 mmol) and TolSCl (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 CH_2Cl_2 and MeOH (×3). The resins in CH_2Cl_2 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 **4a**; 1:2 for **5**)] to give **4a** as a white solid; yield: 19 mg (56%). Linker **5** was also obtained as a white solid: yield: 5 mg (40%). Eluting solvent for TLC: hexane–EtOAc (1:1).

(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 CH_2Cl_2 and MeOH three times to remove excess reactants. Both CH_2Cl_2 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 beads and CH_2Cl_2 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 CH_2Cl_2 (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 CH_2Cl_2, was dissolved in CD_2Cl_2 (0.4 mL) containing (5×10^{-6})% 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 CH_2Cl_2 was mixed with the appropriate sugar derivate **2b–d** (0.3 mmol) and NIS (56 mg, 0.3 mmol). TfOH (22 µL, 0.3 mmol) was added at –40 °C, and the mixture was maintained at –40 °C for 2–3 h. All unreacted reagents were washed out three times with CH_2Cl_2 and MeOH. The resins in CH_2Cl_2 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**)]. **4b** was obtained as a colorless oil; yield: 34 mg (90%). **4c** was also obtained as a colorless oil; yield: 12 mg (31%). **4d** was not obtained. Eluting solvent for TLC: hexane–EtOAc (1:1 for **4b**, 2:1 for **4c** and **4d**).

(17) **Solid-Phase Syntheses of Aminopentyl 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 CH_2Cl_2 (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 CH_2Cl_2 and MeOH. The resin in CH_2Cl_2 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 (2:1 for **7a**, 3:1 for **7b**, 4:1 for **7c**)]. **7a** was obtained as white solid; yield: 19 mg (60%). **7b** was obtained as a colorless oil; yield: 32 mg (93%). **7c** was obtained as a colorless oil; yield 29 mg (82%). Eluting solvent for TLC: hexane–EtOAc (1:2 for **7a**, 1:1 for **7b** and **7c**).

(18) Pathigoolla, A.; Pola, R. P.; Sureshan, K. M. *Appl. Catal., A* **2013**, *453*, 151.

(19) **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. Propargyl bromide (54 µL, 0.6 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–H_2O and CH_2Cl_2. The resulting resin **8** was swollen in CH_2Cl for 2 h. A mixture of resin **8** in 2:2:1 CH_2Cl_2–MeCN–H_2O (2 mL) was treated with azide **9** (79 µL, 0.6 mmol) and $CuSO_4{\cdot}5H_2O$ (154 mg, 0.6 mmol), and the mixture was kept at r.t. for 3 h. $N_2H_4{\cdot}H_2O$ (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 CH_2Cl_2 and MeOH. A mixture of the resin and CH_2Cl_2 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).

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

A 1 M soln of TBAF in THF (500 µL, 0.5 mmol) was added dropwise to a solution of swollen resin **12** (500 mg, 0.1 mmol/g) in CH_2Cl_2, and the mixture was stirred at r.t for 4 h. Unreacted reagents were washed out with CH_2Cl_2 and MeOH to give resin **13**. Resin **13** and a solution of glucopyranoside **2c** (323 mg, 0.5 mmol) in CH_2Cl_2 were mixed with 1-(phenylsulfinyl)piperidine (105 mg, 0.5 mmol) and Tf_2O (82 µL, 0.5 mmol), and the mixture was stirred at –78 °C for 2–3 h. Unreacted reagents were washed out with CH_2Cl_2 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).

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