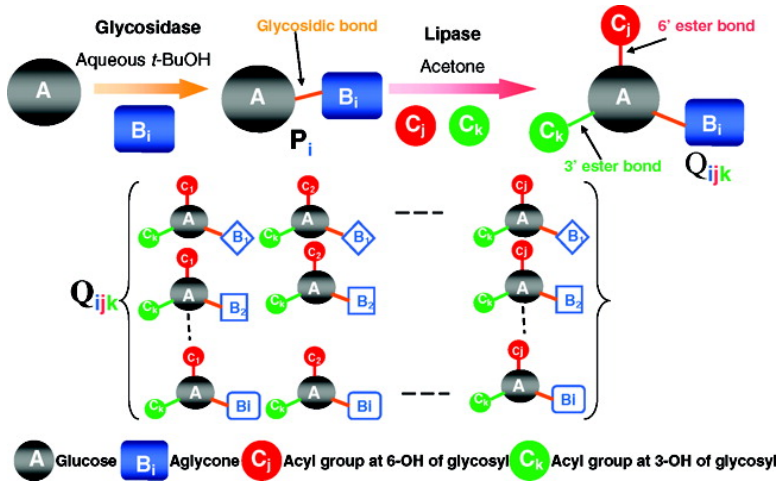
JOURNAL OF combinatorial CHEMISTRY

Article

Assembly of a Three-Dimensional Array of Glycoconjugates by Combinatorial Biocatalysis in Nonaqueous Media

Hui-Lei Yu, Jian-He Xu, Yu-Xiao Wang, Wen-Ya Lu, and Guo-Qiang Lin

J. Comb. Chem., **2008**, 10 (1), 79-87• DOI: 10.1021/cc7001606 • Publication Date (Web): 07 December 2007



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Assembly of a Three-Dimensional Array of Glycoconjugates by Combinatorial Biocatalysis in Nonaqueous Media

Hui-Lei Yu,[†] Jian-He Xu,*[,†] Yu-Xiao Wang,[†] Wen-Ya Lu,[‡] and Guo-Qiang Lin[‡]

State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, P.R. China, and Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, P.R. China

Received September 25, 2007

Glycoconjugates can be artificially synthesized by combinatorial biocatalysis. An example is given in this paper describing the construction of glycoconjugates array by using glycosidase and lipase in nonaqueous media. This array was started from glucose, with three aryl alcohols as the aglycone moiety of glycosides and five acids or esters as acyl donors for combinatorial acylation of glycosides, affording a three-dimensional array containing about 30 members with diverse structures. The array would be more abundant if more aglycones and acyl donors with other structures were filled in. Indeed, diverse classes of carbohydrates besides glucose can also be employed for generating diverse glycoconjugates due to their different roles in numerous physiological responses. The composition and distribution of the demonstration glycoconjugates array was detected and evaluated by HPLC-MS with electrospray ionization. And also, the distribution of the artificial array can be adjusted by changing the molar ratio of the auxiliary materials.

Introduction

Nature's most potent molecules are produced by enzyme-catalyzed reactions, mostly in an aqueous environment. Combinatorial biocatalysis harnesses the natural diversity of enzymatic reactions for the iterative synthesis of organic libraries. These iterative reactions can be performed not only in natural but also in unnatural environments.[1] It should be noted that the breadth of in vitro enzymatic reactions is expanded significantly by using nonaqueous media.[2] Combinatorial biocatalysis is a powerful addition to the expanding array of combinatorial methods created for the generation and optimization of lead compounds in drug discovery and development.[1,3,4] And, combinatorial biocatalysis in nonaqueous media is a useful way for generating more diverse and novel products, which could not be produced by hydrolases under natural aqueous environments due to the limitation of thermodynamic equilibria.

Carbohydrates are abundant in nature, with a wide range of biological roles and many with important medical ramifications.[5] Studies on carbohydrate synthesis and modification by biocatalysis have been pursued vigorously in recent years due to the obvious advantage of the high selectivity of biocatalysts.[4,6,7] Glycosidase can be used for synthesis of glycosides or oligosaccharides via reverse hydrolysis. Lipases were applied to the regioselective acylation of carbohydrates in nonaqueous media.[7] However, there were very few studies[8] reported recently regarding the combinatorial biocatalysis of glycosidase and lipase in nonaqueous media for glycoconjugates preparation starting just from a monosaccharide.

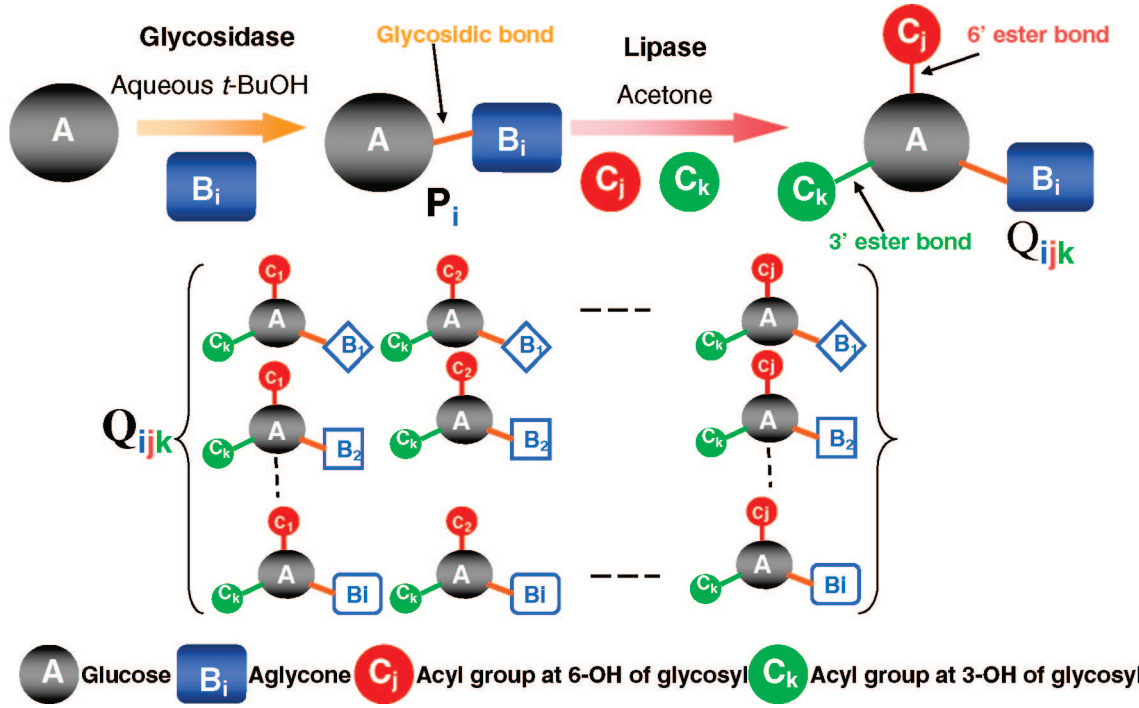
Glucose, one of the most abundant monosaccharides, is a basic block in the material metabolism of conventional biotechnology. Moreover, it is now one of the most important products of biomass degradation and the dominant substrate for biorefinery.[9] Therefore, it was instructive to take glucose as the initial material for preparing a demonstration array of glycoconjugates. The glucose molecule can be used as a scaffold and its hydroxyl groups (OHs) can be connected to diverse chemical blocks. Enzymatic glycosylation by glycosidase and enzymatic acylation by lipase via reverse hydrolysis in nonaqueous media were two efficient approaches to connect aglycones and acyl groups to a backbone material (i.e., glucose), respectively. The aglycone structures range diversely, including mono or oligosaccharides, aryl or alkyl alcohols, peptides, terpenes, phenols, alkaloids, antibiotics, and so on.[10] Various functional acyl donors can also be connected, including not only normal aliphatic acyl donors such as acetic acid, vinyl laurate, vinyl palmitate, and so on[11] but also natural acids with pharmacological properties such as ferulic acid, cinnamic acid, coumaric acid,[12] and even drugs or drug intermediates such as an anti-inflammatory drug (*S*)-ketoprofen. When the glucose molecule was connected with such diverse decorations, a large number of diverse and novel glycoconjugates were generated.

In the present work, in order to greatly diminish the work of independent preparation and screening of each artificially synthesized glycoconjugates, aglycone mixtures and acyl donor mixtures were filled in sequence to prepare an array of glycoconjugates (Scheme 1). This glycoconjugates array contained not only the homogeneous acylation products of each glycoside individually with each acyl donor but also the heterogeneous acylation products of one glycoside with a plural number of different acyl donors at its different OHs.

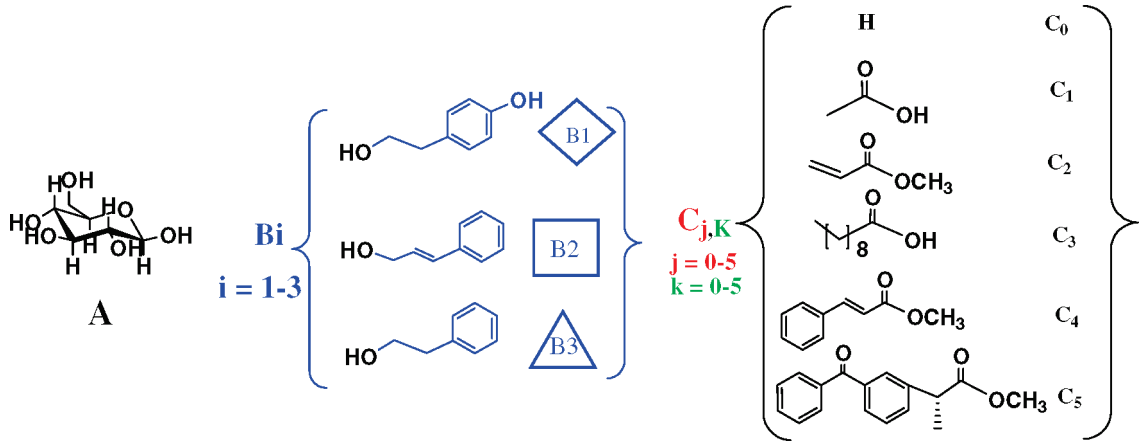
* Corresponding author. E-mail address: jianhexu@ecust.edu.cn. Fax: +86 021 6425 2250.
[†] East China University of Science and Technology.
[‡] Chinese Academy of Sciences.

10.1021/cc7001606 CCC: $40.75
Published on Web 12/07/2007

Scheme 1. Combinatorial Biocatalysis of Glycosidase and Lipase in Nonaqueous Media for Constructing a Three-Dimensional Array of Glycoconjugates (Q_{ijk})



Scheme 2. Materials Used for Assembling a Demonstration Glycoconjugate Array



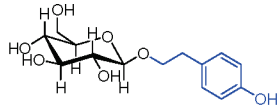
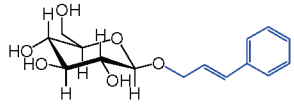
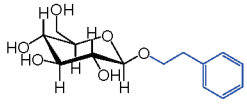
Aglycones can be connected to glucose by glucosidase with absolute regioselectivity, producing their first-step products (P_i). Except for the 1-OH connected to aglycones, other OHs on the glycosyl ring can be acylated. It was shown that enzymatic acylation occurs mainly at 6-OH or 3-OH due to the high regioselectivity of Novozyme 435 (*Candida antarctic* lipase). Therefore, three sites of glycosyl can be connected to different groups, resulting in a three-dimensional (3D) array of diverse glycoconjugates.

In this article, a demonstration glycoconjugate array of glucose was prepared by using three aglycones and five acyl donors as starting materials (Scheme 2). For deconvolution of the large and complicated array, glycoconjugates produced by acylating each glycoside with each acyl donor were first prepared separately. Then, three glycosides in one pot were acylated with one acyl donor and one glycoside was acylated with five acyl donors to form two types of simplified arrays, respectively. On the basis of the analysis of the simplified arrays, the complicated 3D array of glycoconjugates starting from glucose was uncovered gradually.

Results and Discussion

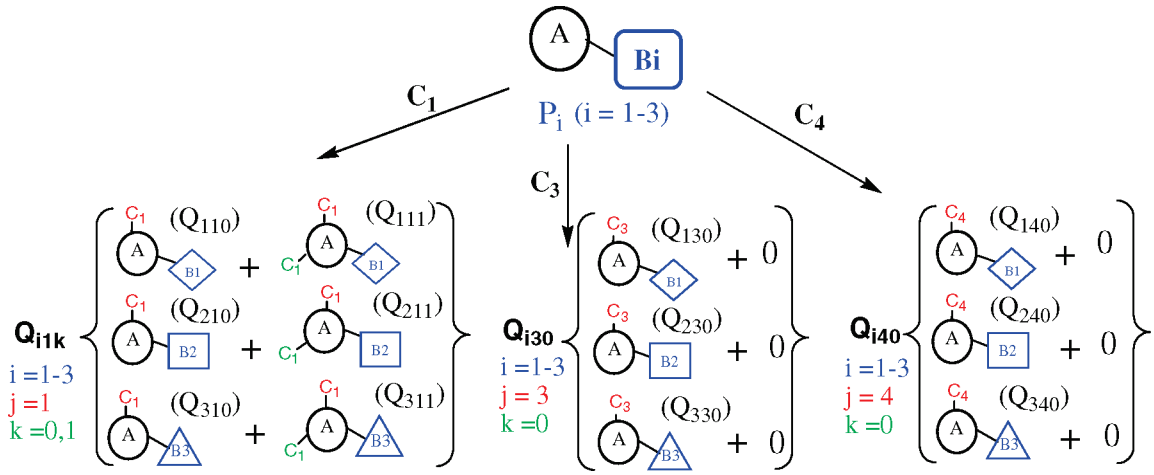
Enzymatic Synthesis of Single Glycoconjugate by Glycosidase and Lipase. Single glycoconjugate was prepared by acylating each of the first-step products (P_1, P_2, and P_3) with each of the acyl donors (C_1−C_5) (Scheme 2). When the acetic acid (C_1) or methyl acrylate (C_2) was used for glycoside acylation, two types of glycosidyl esters were produced, including 6′-*O*-acyl glycosides (Q_{ij0}, $i = 1$–3, $j = 1$–2, $k = 0$) and 3′,6′-*O*-diacyl glycosides (Q_{ijk}, $i = 1$–3, $j = 1$–2, $k = j$). As to caprate acid (C_3), methyl cinnamate (C_4), or (*S*)-ketoprofen methyl ester (C_5), there was mainly one type of product 6′-*O*-acyl glycosides (Q_{ijk}, $i = 1$–3, $j = 3$–5, $k = 0$). The short alkyl acyl group is flexible that was first connected to 6-OH and then further to 3-OH of the glycosyl ring, while the long chain acyl group or aromatic acyl group

Table 1. Enzymatic Synthesis of a Single Glycoconjugate Starting from Glucose[a]

Pi	C_1	C_2	C_3	C_4	C_5
 P_1	Q_{110} (46.1)	Q_{120} (50.3)	Q_{130} (81.9)	Q_{140} (87.4)	Q_{150} (93.3)
	Q_{111} (46.7)	Q_{122} (37.1)			
 P_2	Q_{210} (11.9)	Q_{220} (30.2)	Q_{230} (72.1)	Q_{240} (65.3)	Q_{250} (84.3)
	Q_{211} (66.3)	Q_{222} (25.0)			
 P_3	Q_{310} (19.9)	Q_{320} (9.7)	Q_{330} (62.4)	Q_{340} (48.2)	Q_{350} (78.1)
	Q_{311} (33.9)	Q_{322} (21.7)			

[a] Yields of P_i acylation with Novozyme 435 are presented in parentheses, and the structure of products was determined by NMR (see the Supporting Information).

Scheme 3. One-Pot Acylation of Three Glycosides with One of Acyl Donors C_1, C_3, and C_4



is so space-limiting that it was mainly connected to 6-OH. As to the specific regioselectivity for different types of acyl groups, the number of products in the Q_{ijk} array was less than the probable instances. For example, Q_{ijk} (i = 1–3, j = 0–5, k = 0–5) can be simplified to Q_{ijk} (i = 1–3, j = 1–5, k = 0–2), and the probable number of products was reduced from 108 to 45. Among these 45 probable members, 21 glycoconjugates can be independently synthesized by acylating each of the first-step products (P_i) with each of acyl donors (Table 1), and these 21 compounds can be taken as reference members in further studies.

One-Pot Acylation of Three Glycosides with One Acyl Donor. This array was prepared by three glycosides in one pot acylated with one acyl donor. Three acyl donors (C_1, C_3, and C_4) with typical structures were used, respectively (Scheme 3). When short alkyl acyl donor C_1 was used, it produced a 2D array of Q_{ijk} (i = 1–3, j = 1, k = 0–1), which contains six members (Q_{110}, Q_{111}, Q_{210}, Q_{211}, Q_{310}, Q_{311}). And, there were three members in the array produced by long chain acyl donor C_3 or aromatic acyl donor C_4. On one hand, the acylation degree of the three glycosides was similar regardless of which donor was used (Figure 1). The content of Q_{ijk} (C_{Qijk}) was approximately calculated as follows:

$$C_{Qijk} \approx \frac{A_{Qijk}}{\varepsilon_{Pi}}$$

where C_{Qijk} is the content of Q_{ijk}, A_{Qijk} is the integrated area in HPLC of Q_{ijk}, and ε_{Pi} is the molar extinction coefficient of P_i. The ratio of contents was approximately near 1:1:1 for glycoconjugates derived from the three glucosides. On the other hand, the acylation rate was totally different for acyl donors with diverse structures. For example, the acylation was the fastest with long chain acyl group C_3, being completed within 24 h (Figure 1b). Short alkyl acyl group C_1 was in the next place, and nearly one-quarter of glycosides remained without acylation (Figure 1a). Acyl donors with aryl groups were in the last place, and more than three-quarters of glycosides were unreacted (Figure 1c). Due to the distinct acylation rate of diverse acyl groups, the molar ratio of the acyl groups to be fed in reaction should be adjusted for preparing a balanced array of glycoconjuates containing approximately equal amounts of members.

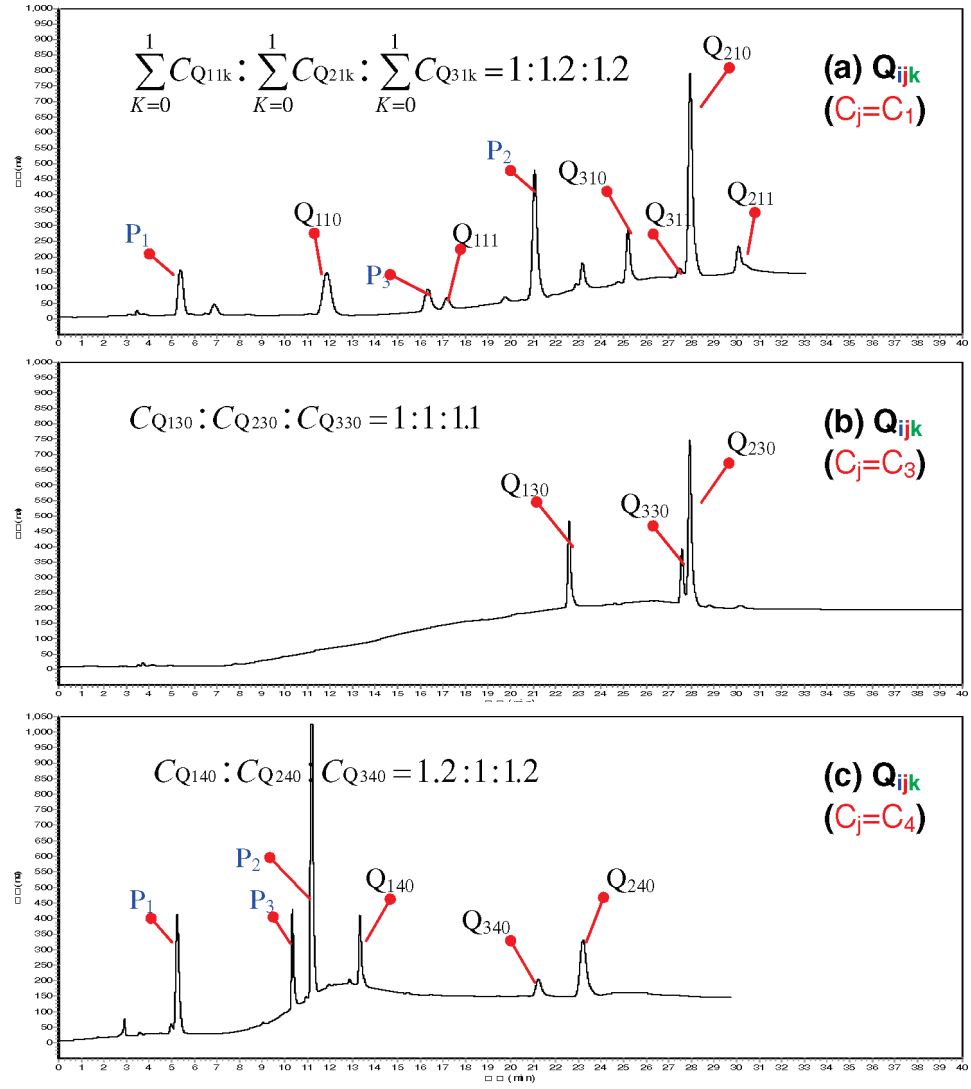
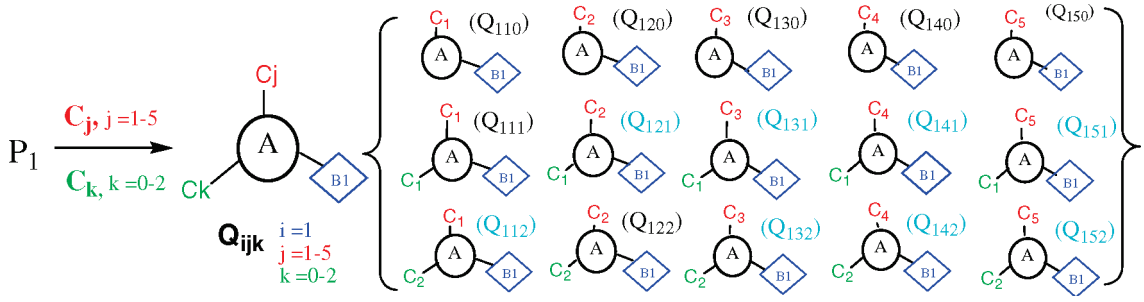


Figure 1. HPLC detection of the arrays of three glycosides acylated with one acyl donor. C_{Qijk} indicates the approximate content of Q_{ijk}. HPLC conditions: C_{18} column eluted at a rate of 0.8 mL·min^{-1}, with an increasing gradient of methanol and water mixture and detected at 215 nm and 30 °C. (a) With acyl donor C_1. The elution gradient values of the methanol and water mixture were as follows: 0−7 min 35/65 v/v; 7−13 min 35/65−45/55; 13−20 min 45/55−60/40; 20−32 min 60/40. (b) With acyl donor C_3. The elution gradient values of the methanol and water mixture were as follows: 0−7 min 60/40; 7−17 min 60/40−90/10; 17−37 min 90/10. (c) With acyl donor C_5. The elution gradient values of the methanol and water mixture were as follows: 0−5 min 35/65−70/30; 5−30 min 70/30.

Scheme 4. Combinatorial Acylation of Glycoside (P_1) with Five Acyl Donors (C_1−C_5) Together in One Pot



One-Pot Acylation of One Glycoside with Five Acyl Donors Together. When the glycoside P_1 was acylated with 5 acyl donors (C_1−C_5) in one pot, a 2D array was produced, in which the 6-OH and 3-OH on the glycosyl ring of each member could be connected to diverse acyl groups (Scheme 4). For preparing an array of glycoconjuates containing approximately equal amount of members, the molar ratio of the acyl groups in one-pot was adjusted to C_1:C_2:C_3:C_4:C_5 = 2:2:1:2:2. First, members of this 2D array were detected by LC-MS (Figure 2). Besides the reference members in Table 1, some new members (i.e., Q_{112}, Q_{121}, Q_{131}) were also formed by the crossover acylation with different acyl donors on two OHs of the glycosyl ring. These new members could be identified by their corresponding mass weights in their mass spectrum and the order of polarity in the HPLC spectrum (Figure

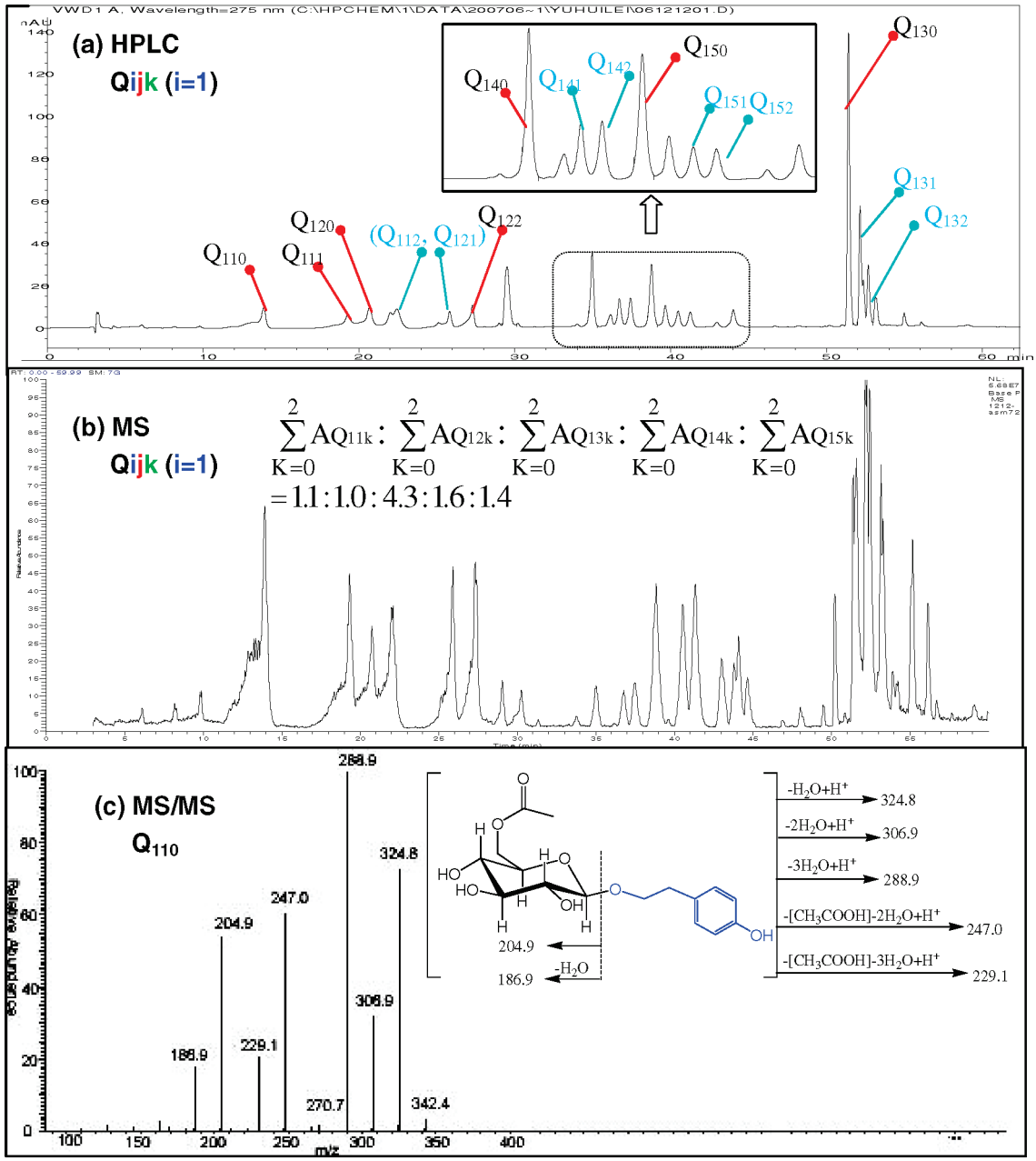


Figure 2. HPLC-MS detection of a 2D array of glycoside P_1 acylated with five acyl donors in one pot. The reference members (Q_{110}, Q_{111}, Q_{120}, Q_{122}, Q_{130}, Q_{140}, Q_{150}) and the new members of the 2D array from P_1 (Q_{112}, Q_{121}, Q_{131}, Q_{132}, Q_{141}, Q_{142}, Q_{151}, Q_{152}) were indicated. The elution order of Q_{112} and Q_{121} in the HPLC spectrum was not clear, and it was supposed that Q_{112} eluted first in the calculation of of their integral area in the HPLC spectrum. HPLC conditions: C_{18} column eluted at a rate of 0.8 mL·min^{-1}, with an increasing gradient of the methanol and water mixture and detected at 275 nm and 30 °C. The elution gradient values of the methanol and water mixture were as follows: 0−18 min 30/70−40/60; 18−30 min 40/60−65/35; 30−40 min 65/35−70/30; 40−50 min 70/30−90/10; 50−60 min 90/10.

2a and 2b). Moreover, they can be verified as the members of the 2D glycoconjugate array since their MS/MS fragmentation behavior was the same with the reference members. This behavior was summarized in Table 2 that the fragment was formed by loss of H_2O, loss of neutral molecules such as CH_3COOH, or loss of aglycones (Figure 2c). Second, the distribution of members with different acyl groups on 6-OH was examined by the ratio of their integral area in HPLC spectrum. The ratio was 1.1:1.0:4.3:1.6:1.4 when the molar ratio of acyl donors (C_1:C_2:C_3:C_4:C_5) was 2:2:1:2:2. The distribution of the 2D array was almost within 1 order of magnitude, and it can be more balanced by further reducing the ratio of C_3. It could be meaningful if this method for detection and evaluation of a 2D array can be applied to the more complicated 3D array of glycoconjugates.

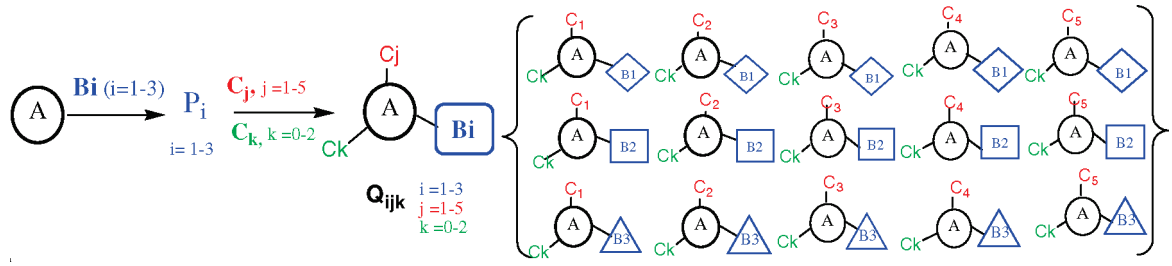
Three-Dimensional Array of Glycoconjugates Starting from Glucose. Finally, a demonstration array of glycoconjugates was constructed with all the starting materials of Scheme 2. From the previous analysis, it was conferred that this demonstration array might contain 45 members (Scheme 5), including two parts: the reference members in Table 1 and the new members produced by the crossover aclyation with different acyl donors. In fact, most of the reference members and 10 of the new members (i.e., Q_{112}, Q_{321}, Q_{351}, Q_{231}) were detected by LC-MS (Figure 3). The newly

Table 2. MS/MS Fragmentation Behavior of Glycoconjugates Derived from Glycoside (P_1)

Q_{ijk} ($i = 1$)	RT (min)	MS	MS/MS fragment
Q_{110}	13.96	360.07 (M + NH_4^+)	324.8 (M − H_2O + H^+), 306.9 (M − $2H_2O$ + H^+), 288.9 (M − $3H_2O$ + H^+); 204.9 (GluAc + H^+), 186.9 (GluAc − H_2O + H^+); 247.0 (M − [CH_3COOH] − $2H_2O$ + H^+), 229.1 (M − [CH_3COOH] − $3H_2O$ + H^+)
Q_{111}	19.35	402.03 (M + NH_4^+)	246.8 (Glu2Ac + H^+), 288.8 (M − [CH_3COOH] − $2H_2O$ + H^+)
Q_{120}	20.73	372.26 (M + NH_4^+)	336.7 (M − H_2O + H^+), 318.8 (M − $2H_2O$ + H^+), 300.9 (M − $3H_2O$ + H^+); 216.9 (GluAl + H^+), 199.0 (GluAl − H_2O + H^+); 247.0 (M − [$CH_2CHCOOH$] − $2H_2O$ + H^+), 229.0 (M − [$CH_2CHCOOH$] − $3H_2O$ + H^+)
Q_{122}	27.33	431.13 (M + Na^+)	358.9 (M − [$CH_2CHCOOH$] + Na^+)
Q_{140}	35.03	453.18 (M + Na^+)	nd
Q_{150}	38.82	559.18 (M + Na^+)	nd
Q_{130}	51.60	477.28 (M + Na^+)	459.7 (M − H_2O + Na^+)
Q_{112}/Q_{121}[a]	22.13	414.00 (M + NH_4^+)	378.8 (M − H_2O + H^+), 258.9 (GluAcAl + H^+)
Q_{121}/Q_{112}	25.84	414.00 (M + NH_4^+)	379.1 (M − H_2O + H^+), 258.9 (GluAcAl + H^+); 229.1 (M − [$CH_2CHCOOH$] − [CH_3COOH] − $2H_2O$ + H^+)
Q_{141}	36.80	495.09 (M + Na^+)	435.3 (M − [CH_3COOH] + Na^+)
Q_{142}	37.48	507.15 (M + Na^+)	435.2 (M − [$CH_2CHCOOH$] + Na^+)
Q_{151}	40.55	601.04 (M + Na^+)	541.3 (M − [CH_3COOH] + Na^+)
Q_{152}	41.34	613.19 (M + Na^+)	541.2 (M − [$CH_2CHCOOH$] + Na^+)
Q_{131}	52.50	519.30 (M + Na^+)	459.3 (M − [CH_3COOH] + Na^+)
Q_{132}	53.17	531.28 (M + Na^+)	459.5 (M − [$CH_2CHCOOH$] + Na^+)

[a] The elution order of Q_{112} and Q_{121} in the HPLC spectrum was not clear.

Scheme 5. Three-Dimensional Array of Glycosides P_i ($i = 1-3$) Acylated with Acyl Donors C_{jk} ($j = 1-5$, $k = 0-2$) in One Pot



produced members were also verified by their MS/MS fragmentation behavior. The distribution of each dimension was calculated as follows:

i-Dimension

$$D_i = \sum_{j=1}^{5}\sum_{k=0}^{2} C_{Q1jk} : \sum_{j=1}^{5}\sum_{k=0}^{2} C_{Q2jk} : \sum_{j=1}^{5}\sum_{k=0}^{2} C_{Q3jk}$$

j-Dimension

$$D_j = \sum_{i=1}^{3}\sum_{k=0}^{2} C_{Qi1k} : \sum_{i=1}^{3}\sum_{k=0}^{2} C_{Qi2k} : \sum_{i=1}^{3}\sum_{k=0}^{2} C_{Qi3k} : \sum_{i=1}^{3}\sum_{k=0}^{2} C_{Qi4k} : \sum_{i=1}^{3}\sum_{k=0}^{2} C_{Qi5k}$$

k-Dimension

$$D_k = \sum_{i=1}^{3}\sum_{j=1}^{5} C_{Qij0} : \sum_{i=1}^{3}\sum_{j=1}^{5} C_{Qij1} : \sum_{i=1}^{3}\sum_{j=1}^{5} C_{Qij2}$$

When the molar ratio of acyl donors (C_1:C_2:C_3:C_4:C_5) was 2:2:1:2:2 (Figure 3a), it was within 1 order of magnitude for the *i*-dimension and *j*-dimension, while it was unbalanced for the *k*-dimension in that the members with two acyl groups were two less than that with one acyl group. This situation can be improved by increasing the molar ratio of C_1 and C_2 in acyl donors. When the ratio was adjusted to 8:8:1:4:4 (Figure 3b), a more balanced array was produced. The relative approximate content (C_{Qijk}) of each member of Q_{ijk} (C_1:C_2:C_3:C_4:C_5 = 8:8:1:4:4) was shown in Figure 4.

Conclusions

In summary, a glycoconjugate array can be constructed by combinatorial biocatalysis of glycosidase and lipase in nonaqueous media. In this article, a demonstration glycoconjugate array of glucose was prepared by using three aglycones and five acyl donors as starting materials. The composition and distribution of the demonstration glycoconjugates array were analyzed and evaluated by LC-MS, and the members produced by the crossover acylation were verified by their MS/MS fragmentation behavior. The distribution of the array can be adjusted by changing the molar ratio of acyl donors. The constructed array contained about 30 members with diverse structures, and some of them are being assayed for neural pharmacological activity.

This array would be more abundant if more aglycones and acyl donors with other structures were introduced. Indeed, diverse classes of carbohydrates besides glucose can be employed for generating diverse glycoconjugates due to their different roles in numerous physiological responses.[13] Methods for detection and evaluation of the demonstration glycoconjugates array can be applied to study the larger glycoconjugate library.

Moreover, it was meaningful by mixing the acyl groups in one pot for discovering useful glycoconjugates. On one hand, there were more products in glycoconjugate array

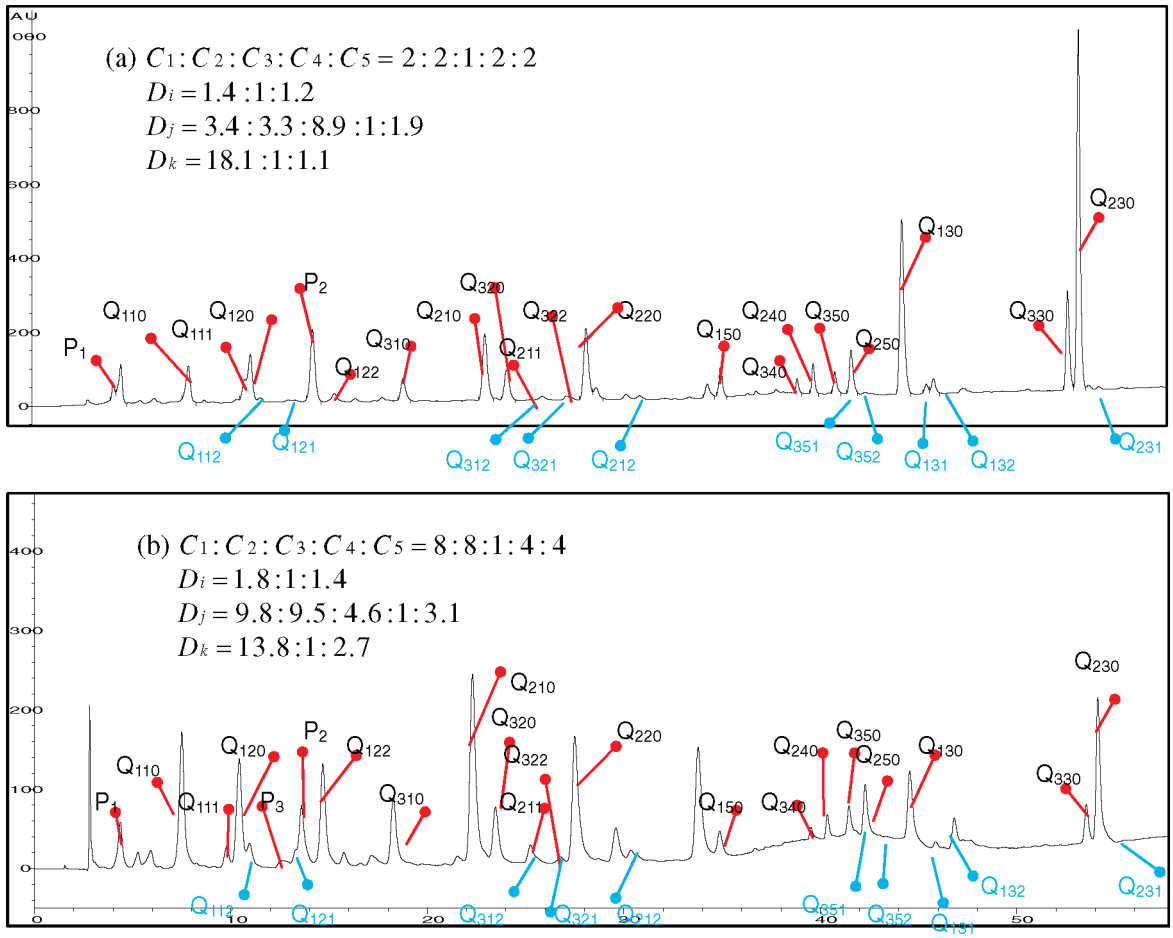


Figure 3. HPLC-MS detection of the demonstration 3D array of glycoconjugates starting from glucose (only HPLC spectra were shown here while the mass spectrum is shown in the Supporting Information). (a) C_1:C_2:C_3:C_4:C_5 = 2:2:1:2:2 (C_3, 50 mmol·L^{-1}). (b) C_1:C_2:C_3:C_4:C_5 = 8:8:1:4:4 (C_3, 25 mmol·L^{-1}). HPLC conditions: C_{18} column eluted at a rate of 0.8 mL·min^{-1}, with an increasing gradient of the methanol–water mixture and detected at 275 nm and 30 °C. The elution gradient values of the methanol–water mixture were as follows: 0−10 min 40/60−50/50; 10−30 min 50/50−65/35; 30−35 min 65/35−80/20; 35−40 min 80/20; 40−55 min 95/5.

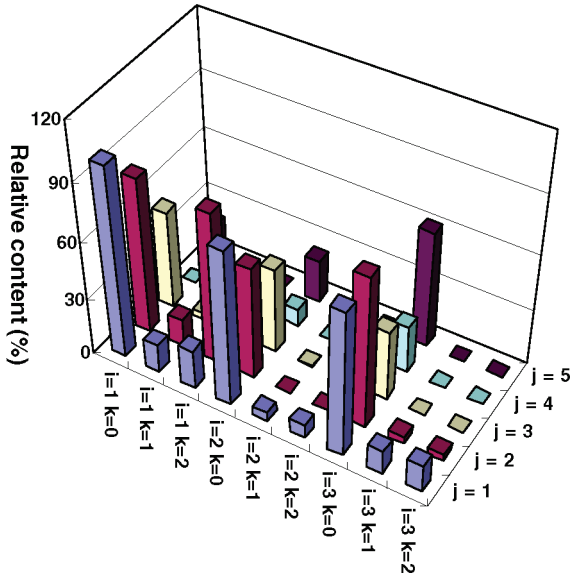


Figure 4. Relative contents (C_{Qijk}) of members in the demonstration glycoconjugate array Q_{ijk} (i = 1–3, j = 1–5, k = 0–2). The content of Q_{110} was taken as 100%.

prepared by mixing the starting materials in one pot than in the library composed of glycoconjugates respectively synthesized with each starting material. On the other hand, both the work of preparation and that of screening can be diminished greatly. And, the active member can be identified from the complicated array by the method of deconvolution in combinational chemistry.[14] By combinatorial biocatalysis with glycosidase and lipase, a large quantity of natural and unnatural glycoconjugates can be produced very easily. This represents a powerful addition to the expanding array of combinatorial chemistry.

Experimental Section

Materials. Tyrosol was purchased from Tianxun Co. (Nanjing, China), phenethyl alcohol was from Acoros Co., and cinnamyl alcohol was from Shuangxi Co. (Shanghai, China). Acyl donors and all the solvents were commercial reagents of analytical grade. Novozyme 435 (LC2000206) was purchased from Novo-nordisk (China). Apple seeds were taken from a fruit processing plant as a waste, and the apple seed meal was prepared as described previously.[15,16]

Glycosylation via Reverse Hydrolysis. Glucosides were enzymatically synthesized with the aid of β-glucosidase extracted from apple seed in a mixed medium consisting of *tert*-butanol and phosphate buffer (Na_2HPO_4/KH_2PO_4, 0.067 mol·L^{-1}, pH 6.0).[16] The products were purified by flash chromatography on silica gel (eluent: EtOAc/CH_3OH = 13/1, v/v).

Synthesis of Single Glycoconjugates. To 10 mL acetone were added 25 mmol·L^{-1} glycoside (P_i), 250 mmol·L^{-1} acyl donor, 2 g molecular sieve 3Å, and 200 mg Novozyme 435. The flask was sealed and stirred at 50 °C for 72 h. Then, the reaction mixture was filtered, and the filtrate was evaporated under reduced pressure. The obtained syrup was purified by flash chromatography on silica gel (eluent: EtOAc/petroleum ether = 4/1−1/1, v/v).

One-Pot Acylation of Three Glycosides with One Acyl Donor. In the acetone system, P_1, P_2, and P_3 (25 mmol·L^{-1} each) were added into one pot and 250 mmol·L^{-1} of the acyl donor, 200 mg·mL^{-1} of molecular sieve 3Å, and 20 mg·mL^{-1} of Novozyme 435 were also added. The flask was sealed and stirred at 50 °C for 24 h. Then the reaction mixture was filtered, and the filtrate was evaporated under reduced pressure. The resultant syrup was purified by flash chromatography on silica gel. The eluant of EtOAc/petroleum ether (1/1, v/v) was first used to remove the excessive acyl donor, and then, the eluant of EtOAc/CH_3OH = 9/1 (v/v) was used for product extraction. This fraction was evaporated under reduced pressure, and the residual syrup was dissolved with CH_3OH. The solution was filtered through a 0.45 mm PTFE syringe filter, and a 10 μL aliquot of the filtrate was injected into the LC-MS system for analysis.

One-Pot Acylation of One Glycoside with Five Acyl Donors. Into the acetone system in one pot were added five acyl donors including acetic acid (C_1, 100 mmol·L^{-1}), methyl acrylate (C_2, 100 mmol·L^{-1}), caprate acid (C_3, 50 mmol·L^{-1}), methyl cinnamate (C_4, 100 mmol·L^{-1}), and (*S*)-ketoprofen methyl ester (C_5, 100 mmol·L^{-1}). And, salidroside (P_1, 25 mmol·L^{-1}), molecular sieve 3Å (200 mg·mL^{-1}), and Novozyme 435 (20 mg·mL^{-1}) were also added. The flask was sealed and stirred at 50 °C for 72 h. The sample preparation process for this array was the same as above.

Combinatorial Acylation of Three Glycosides with Five Acyl Donors in One Pot. Three glucosides were enzymatically synthesized with the aid of β-glucosidase from apple seed in a mixed medium consisting of *tert*-butanol and phosphate buffer, and the first-step products thereof were purified by flash chromatography on silica gel (eluent: EtOAc/CH_3OH = 13/1, v/v).[16] Into the acetone system, five acyl donors with a certain molar ratio, including acetic acid (C_1), methyl acrylate (C_2), caprate acid (C_3), methyl cinnamate (C_4), and (*S*)-ketoprofen methyl ester (C_5), and three glycosides, the first-step products, including salidroside (P_1), rosin (P_2), and phenylethyl glucopyranoside (P_3), were added, before molecular sieve 3Å (200 mg·mL^{-1}) and Novozyme 435 (20 mg·mL^{-1}) were finally added. The flask was sealed and stirred at 50 °C for 72 h. The sample preparation process for this 3D array was the same as above.

HPLC, LC-MS, and MS/MS Detection. HPLC-UV analyses were performed using SPD-10A vp (Shimadzu) with a column of C_{18} (Ø 5.0 mm × 200 mm, 5 μm). LC-MS analyses were performed using an Agilent 1100 Series HPLC system (Agilent Technologies Co., USA) with a UV detector, and the separation used was a Zorbax SB-C18 column (250 mm × 4.6 mm, 5 μm) with a Zorbax SB-C18 guard column (12.5 mm × 4.6 mm, 5 μm). Mass spectra were acquired using a LCQ Deca XP ion trap mass spectrometer (ThermoFinnigan, USA) equipped with an ESI source. Nitrogen was used as the sheath and auxiliary gas, and helium was used as the damping and collision gas. All the mass spectra were acquired in the positive ion mode with ion spray voltage at 4.8 kV, capillary temperature at 300 °C, capillary voltage at 15 V, sheath gas flow rate at 50 (arbitrary units), auxiliary gas flow rate at 15 (arbitrary units), and tube lens offset at 15 V. The first-order mass spectra were recorded in the range *m*/*z* 200–1000. The relative collision energy was 35% of maximum to produce optimum yields of fragment ions.

General Methods. Optical rotations were measured using a Jasco P-1030 polarimeter; NMR was recorded on a Bruker AM-300 spectrometer or a Bruker DRX-500 spectrometer; Mass spectra were recorded on an LCQ Deca Xp (Thermo Finnigan) using ESI mode. FT-IR analysis was recorded on a Bio-Rad FTS-185.

Selected Analytical Data (Q_{110}). $[\alpha]_D^{25}$ −25.9 (*c* 0.5, CH_3OH); ^{1}H NMR (300 MHz, CD_3OD) δ/ppm 2.04 (s, 3H), 2.82 (dd, $J_1 = J_2 = 6.6$ Hz, 2H), 3.17 (t, $J = 7.8$ Hz, 1H), 3.26–3.32 (m, 1H), 3.32–3.38 (m, 1H), 3.41–3.47 (m, 1H), 3.65–3.73 (m, 1H), 3.89–3.98 (m, 1H), 4.20 (dd, $J_1 = 12.0$ Hz, $J_2 = 6.0$ Hz, 1H), 4.29 (d, $J = 8.1$ Hz, 1H), 4.38 (dd, $J_1 = 12.3$ Hz, $J_2 = 2.4$ Hz, 1H), 6.69 (d, $J = 11.1$ Hz, 2H), 7.05 (d, $J = 8.4$ Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ/ppm 21.0, 36.7, 65.1, 71.9, 72.6, 75.3, 75.5, 78.2, 104.8, 116.4, 131.0, 131.2, 157.2, 173.1; ESI MS (*m*/*z*): ($M + NH_4^+$) 360.1. For more information, see the Supporting Information.

Acknowledgment. This research was financially supported by the National Natural Science Foundation of China (Grant Nos. 203900506 and 20672037) and the Ministry of Science and Technology, P.R. China (Grant No. 2003CB716008).

Supporting Information Available. HPLC-MS detection of the demonstration 3D array of glycoconjugates starting from glucose (MS spectrum) and compound data including ^{1}H NMR, ^{13}C NMR, ESI MS, and so on. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Dordick, J. S.; Khmelnitsky, Y. L.; Sergeeva, M. V. *Curr. Opin. Microbiol.* **1998**, *1*, 311–318.

(2) (a) Dordick, J. S. In *Applied Biocatalysis*; Clark, D. S., Blanch, H. W., Eds.; Marcel Dekker Co.: New York, 1990; Vol. 1, pp 1–51. (b) Klibanov, A. M. *Trends Biotechnol.* **1997**, *15*, 97–101. (c) Benjamin, G. D.; Viviane, B. *Nat. Prod. Rep.* **2001**, *18*, 618–640. (d) Krishna, S. H. *Biotechnol. Adv.* **2002**, *20*, 239–267.

(3) (a) Michels, P.; Khmelnitsky, Y. L.; Dordick, J. S.; Clark, D. S. *Trends Biotechnol.* **1998**, *16*, 210–215. (b) Altreuter, D. H.; Clark, D. S. *Curr. Opin. Biotechnol.* **1999**, *10*, 130–136. (c) Krstenansky, J. L.; Khmelnitsky, Y. L. *Bioorg. Med. Chem.* **1999**, *7*, 2157–2162. (d) Rich, J. O.; Michels, P. C.; Khmelnitsky, Y. L. *Curr. Opin. Chem. Biol.* **2002**, *6*, 161–167. (e) Cipolla, L. *Comb. Chem. High Throughput Screening* **2004**, *7*, 101–114. (f) Wilkinson, B.; Bachmann, B. O. *Curr. Opin. Chem. Biol.* **2006**, *10*, 169–176.

(4) Riva, S. *Curr. Opin. Chem. Biol.* **2001**, *5*, 106–111.

(5) (a) Varki, A. *Glycobiology* **1993**, *3*, 97–l30. (b) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683–720. (c) McAullffe, J. C.; Hindsgaul, O. *Chem. Ind.* **1997**, *5*, 170–l74. (d) McGarvey, G. J.; Wong, C. H. *Liebigs Ann. Recl.* **1997**, 1059–l074.
(6) (a) Wong, C. H.; Whitesides, G. N. *Enzymes in Synthetic Organic Chemistry*; Elsevier Science Ltd: Oxford, 1994; pp 252–311. (b) Thiem, J. *FEMS Microbial Rev.* **1995**, *16*, 193–211. (c) Wong, C. H.; Halcomb, R. L.; Lchikawa, Y.; Kajimoto, T. *Angew. Chem., Int. Ed.* **1995**, *34*, 412–432.
(7) Weber, L. *Curr. Opin. Chem. Biol.* **2000**, *4*, 295–302.
(8) Otto, R.; Ornscheuer, U. T.; Sykdatk, C.; Schmid, R. D. *Biotechnol. Lett.* **1998**, *20*, 437–440.
(9) (a) Bayer, E. A.; Lamed, R.; Himmel, M. E. *Curr. Opin. Biotechnol.* **2007**, *18*, 237–245. (b) Sanders, J.; Scott, E.; Weusthuis, R.; Mooibroek, H. *Macromol. Biosci.* **2007**, *7*, 105–117.
(10) (a) Crout, D. H. G.; Vic, G. *Curr. Opin. Chem. Biol.* **1998**, *2*, 98–111. (b) Scigelova, M.; Singh, S.; Crout, D. H. G. *J. Mol. Catal. B: Enzym.* **1999**, *6*, 483–494. (c) Yu, H. L.; Xu, J. H.; Lin, G. Q. *Chin. J. Org. Chem.* **2006**, *8*, 1052–1058.
(11) (a) Kontogianni, A.; Skouridou, V.; Sereti, V.; Stamatis, H.; Kolisis, F. N. *J. Mol. Catal. B: Enzym.* **2003**, *21*, 59–62. (b) Ferrer, M.; Cruces, M. A.; Plou, F. J.; Bernabe, M.; Ballesteros, A. *Tetrahedron* **2000**, *56*, 4053–4061.
(12) Riva, S. *J. Mol. Catal. B.: Enzym.* **2002**, *19–20*, 43–54.
(13) (a) Roseman, S. *J. Biol. Chem.* **2001**, *276*, 41527–41542. (b) Koeller, K. M.; Wong, C. H. *Chem. Rev.* **2000**, *100*, 4465–4493.
(14) de la Gôutte, J. T.; Khan, J. A.; Vulfson, E. N. *Biotechnol. Bioeng.* **2001**, *75*, 93–99.
(15) Yu, H. L.; Xu, J. H.; Lu, W. Y.; Lin, G. Q. *Enzyme Microb. Technol.* **2007**, *40*, 354–361.
(16) Tong, A. M.; Lu, W. Y.; Xu, J. H.; Lin, G. Q. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2095–2097.

CC7001606