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Programmable one-pot synthesis of tumor-associated carbohydrate antigens Lewis X dimer and KH-1 epitopes

Bin-Ling Tsai [a,b], Jeng-Liang Han [a], Chien-Tai Ren [a], Chung-Yi Wu [a,*], Chi-Huey Wong [a,b,*]

[a] Genomics Research Center, Academia Sinica, Taiwan
[b] Department of Chemistry, National Taiwan University, Taipei, Taiwan

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Dedicated to Professor Harry Wasserman on the occasion of his 90th birthday

ABSTRACT

The total synthesis of the antigenic Lewis X (Le^x) dimer and KH-1 epitopes by a reactivity-based programmable one-pot synthetic strategy is reported. This approach can minimize the protection–deprotection and purification steps. Using the reactivity-based one-pot synthetic method, the fully protected Lewis X (Le^x) dimer and KH-1 epitopes were furnished in a facile manner, which were globally deprotected to give the Le^x dimer and KH-1 epitopes.

Lewis X (Le^x) dimer and KH-1, isolated by Hakomori in 1986,[1] are tumor-associated carbohydrate antigens and considered as markers of colonic adenocarcinoma.[1,2] They can be used to develop carbohydrate-based anticancer vaccines and carbohydrate microarray for diagnosis. Le^x dimer has been synthesized via stepwise [3+2+3] or [3+3+2] strategies,[3–6] soluble polymer supported synthesis,[7] orthogonal, and iterative methods.[8,9] Chemical synthesis of KH-1 was first reported by both Schmidt[10] and Danishefsky[11] independently in 1997. In 2004, Seeberger used the automated solid-phase synthesis to construct KH-1.[12] Stepwise [4+3] strategy was used by Danishefsky[13] and Boons[14] independently in 2005.

Our group has developed a programmable one-pot strategy for the synthesis of complex oligosaccharides.[15–21] The methodology is based on the use of designer thioglycoside building blocks with defined relative reactivity values (RRVs) that are collected in the Optimer database.[22] Herein, we report the use of this strategy to prepare antigens Le^x dimer and KH-1 epitopes.

From the structural point of view, the subtle difference between the epitope structure of dimeric $Lewis^X$ (**1**) and KH-1 (**2**) is that the latter has an extra L-fucose with an α(1→2) linkage to the nonreducing end galactose moiety (the $Lewis^Y$ epitope). Accordingly, we designed a convergent synthetic approach utilizing a reactivity-based one-pot methodology (Scheme 1), in which **1** could be derived from the building blocks **3**,[22], **4**,[18] and **6** by one-pot glycosylation. In a similar manner, **2** could be prepared from the corresponding building blocks **3**, **5**,[18] and **6**. The disaccharides **4** and **5** could be prepared from thiogalatosides **7**[22] and **8**,[22] respectively, with **9**.[22] The reducing end trisaccharide building block **6** in turn could be assembled from building blocks **3**, **10** (RRV = 115),[9] and **11** via a second one-pot glycosylation.

To synthesize building blocks **4** and **5** (Scheme 2), we started by treating thiogalactoside **7** (RRV = 5200) with **9** (RRV = 282) in the presence of *N*-iodosuccinimide (NIS) and 0.1 equiv of triflic acid (TfOH) in CH_2Cl_2 at −35 °C to give disaccharide **12** in 43% yield. The levulinate (Lev) protecting group in **9** seems to be critical for increasing the yield. Replacement of the levulinoyl group with the benzoyl group resulted in a substantial decrease in product yield. Selective deprotection of the Lev ester in **12** by treatment with hydrazine hydrate in an acetic acid/pyridine mixture afforded building block **4** in 98% yield. Likewise, building block **5** was prepared from glycosylation of **8** (RRV = 4000) and **9** in a comparable yield. The synthesis of **11** was started from the known glycosyl bromide **14**.[23] Treatment of **4** with azido alcohol **15** in the presence of iodine and DDQ[24] gave **16** exclusively in 71% yield. Deacetylation of **16** followed by benzylidene formation between C4–OH and C6–OH afforded **17** (91% for two steps). Compound **17** was converted into **11** by a three step sequence: protecting C3–OH in **17** as Lev ester, followed by reductive benzylidene ring cleavage and selective Lev deprotection.

Next, we proceeded to the assembly of trisaccharide building block **18** via a one-pot sequential glycosylation (Scheme 3). To take advantage of the reactivity difference between C3–OH and C4–OH in building block **11**[25], thiogalactoside **10** was chosen to be the first glycosyl donor in order to introduce the galactose moiety in a desired regioselective manner. In a separate experiment, treatment of **11** with glycosyl donor **10** in the presence of NIS and a catalytic amount of TfOH in CH_2Cl_2 at −40 °C gave two regioisomers in a ratio of 10–1, in which the C4-isomer was obtained as the major product. Encouraged by these results, we further investigated the one-pot synthesis of **18**. Building block **11** (1.0 equiv) was coupled

* Corresponding authors.
E-mail addresses: cyiwu@gate.sinica.edu.tw (C.-Y. Wu), chwong@gate.sinica.edu.tw (C.-H. Wong).

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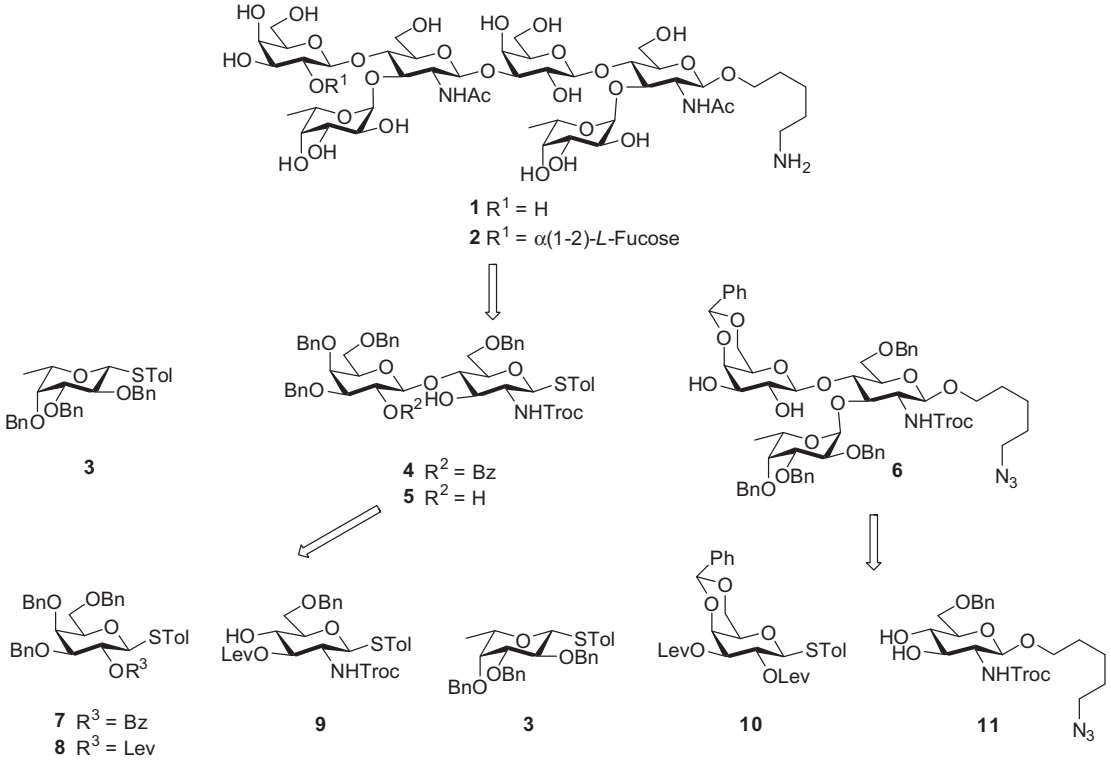
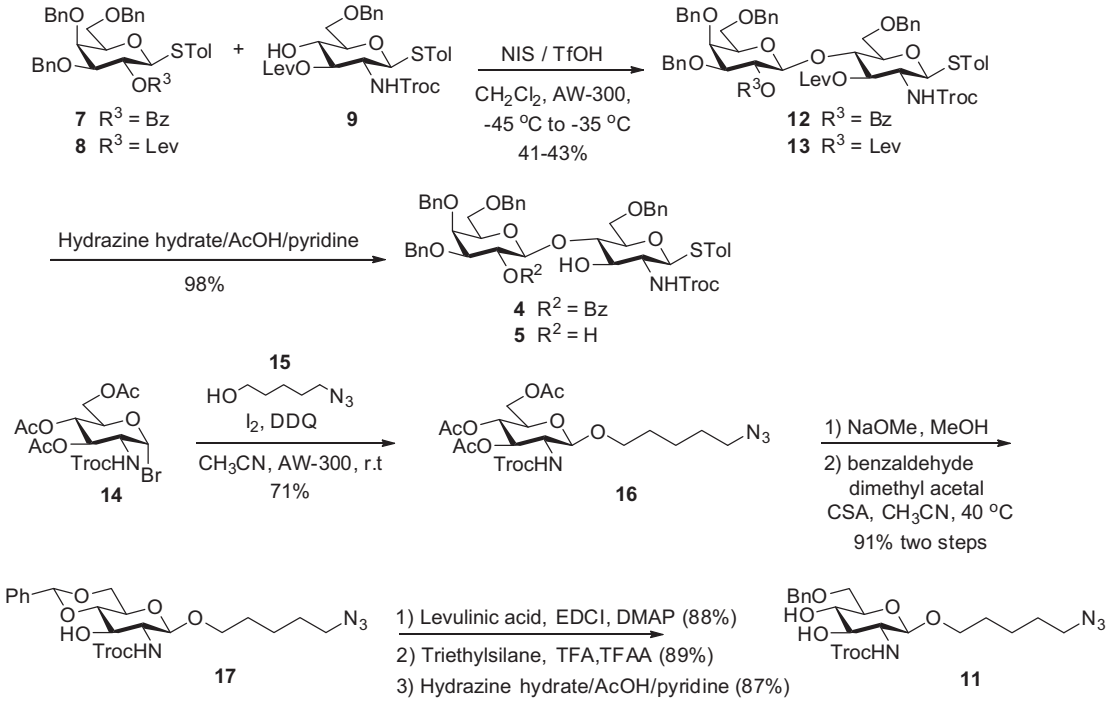
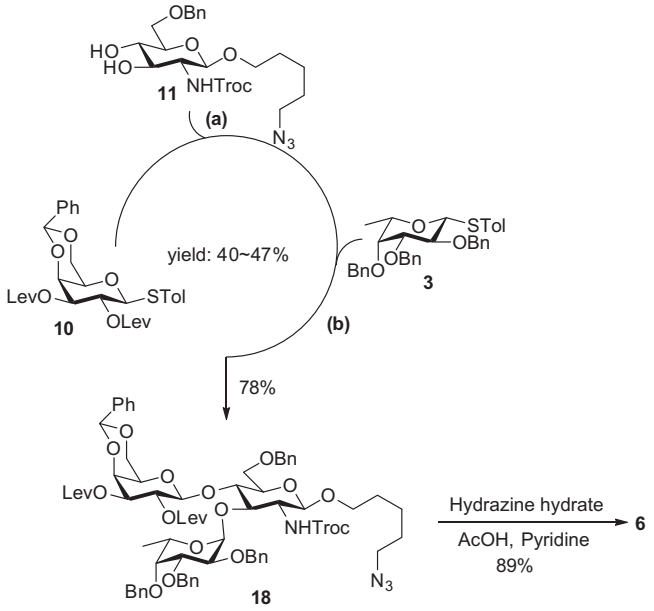


Scheme 1. Retro-synthetic analysis of Le^x dimer **1** and KH-1 **2**.

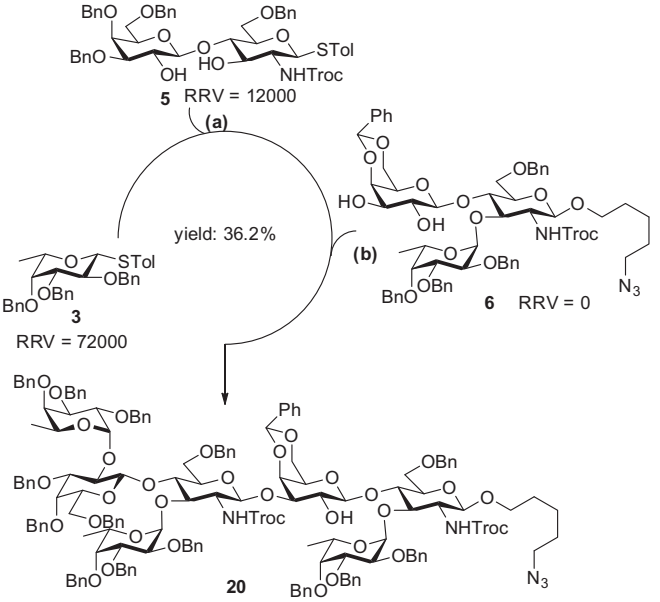


Scheme 2. Synthesis of building blocks **4**, **5** and **11**.

with **10** (1.4 equiv) in the presence of NIS/TfOH (0.1 equiv) at −40 °C. After the complete consumption of **11** was confirmed by TLC, the temperature was cooled to −50 °C, and perbenzylated fucosyl building block **3** (1.2 equiv) was added, followed by the addition of NIS/TfOH. After the complete consumption of **3** was confirmed by TLC analysis, trisaccharide **18** was obtained in 40–47% yield after standard work up and purification and no regioisomer of **18** was detected. Building block **6** was then prepared by selective Lev deprotection of **18** under the conditions of hydrazine hydrate in an acetic acid/pyridine mixture.



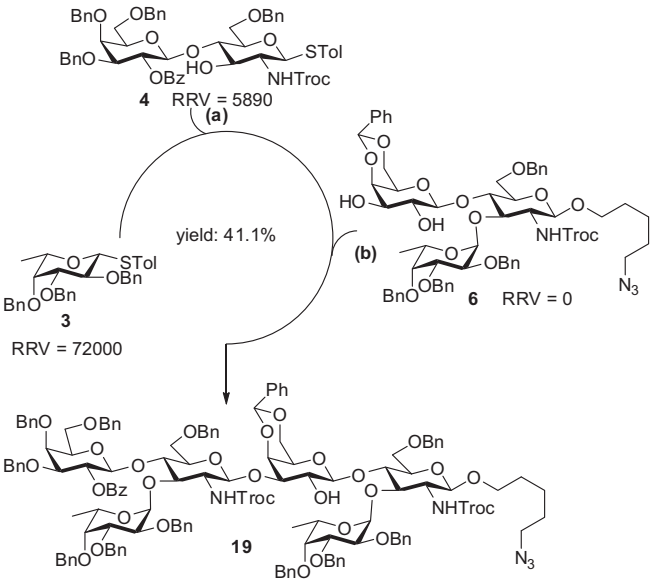
Scheme 3. One-pot synthesis of trisaccharide building block **18**.



Scheme 5. One-pot synthesis of fully protective KH-1 epitope.

With all the desired building blocks in hand, we examined the feasibility of one-pot synthesis of compounds **1** and **2**. First, the reaction of glycosyl donor **3** with acceptor **4** and **5**, respectively, was performed under various conditions in order to optimize the stereochemical outcome. It was found that the best result was obtained when the reaction was carried out in CH_2Cl_2 at −45 °C and activated by NIS/TfOH. The reaction temperature seems to be crucial for the success of this reaction; little products with poor anomeric selectivity and by-products derived from succinimde were observed when the reaction was carried out at lower temperature, such as −78 °C.

For the reducing end trisaccharide **6**, there was a distinct acceptor reactivity bias where C3–OH>>C2–OH was speculated for the reason of their steric accessibility.[9] With these preliminary results in hand, we turned our attention to the one-pot synthesis of hexasaccharide **19**, a precursor of **1** in a [1+2+3] fashion (Scheme 4). Perbenzylated fucosyl building block **3** (1.1 equiv, RRV = 7.2×10^4) was coupled with the less reactive disaccharide building block **4** (1.0 equiv, RRV = 5890) in the presence of NIS/TfOH at −45 °C. After the complete consumption of **3** was confirmed by TLC analysis, the reaction temperature was raised to −35 °C, and trisaccharide acceptor **6** (0.8 equiv, RRV = 0) was added, followed by the addition of NIS/TfOH. The second glycosylation was generally completed in 1 h at −35 °C as demonstrated by TLC analysis. The reaction was then worked up by the standard procedures to provide **19** in 41% yield (based on **6**) after flash silica gel chromatography (EA/Hexane = 1:2). Following the same reaction sequence, heptasaccharide **20**, a precursor of **2** was prepared in a [1 × 2+2+3] fashion from corresponding building blocks **3** (2.2 equiv, RRV = 7.2×10^4), **5** (1.0 equiv, RRV = 1.2×10^4), and **6** (0.6 equiv, RRV = 0) in 36% yield (based on **6**) (Scheme 5).



Scheme 4. One-pot synthesis of fully protective Le^x dimer epitope.

To complete the synthesis, compounds **19** and **20** were subjected to global deprotection (Scheme 6). The process started with the removal of the Acyl and Troc protecting groups by alkali hydrolysis (1 N NaOH in THF at 50 °C for 24 h), followed by reacetylation of the exposed hydroxyl and amine functionalities with acetic anhydride in pyridine, and O-deacetylation with NaOMe in methanol to provide intermediate **21** and **22**, respectively, in 56–62% yield. The rest of the protecting groups in **21** and **22** were removed by catalytic hydrogenolysis (H_2, 20% $Pd(OH)_2/C$, in MeOH/H_2O/AcOH) to afford the target molecules **1** and **2**, respectively, in 61–80% yield.

This approach extensively utilizes the diol functionality of the building blocks by introducing two of the same carbohydrate units simultaneously or taking the advantages of the distinct reactivity order of these hydroxyl groups for attachment of two different carbohydrates in a regioselective manner. Thus, the protection–deprotection steps are minimized, and we present a simple, elegant, convergent, and highly efficient reactivity-based programmable one-pot synthesis of Le^X dimer **1** and KH-1 **2** epitopes.

Acknowledgment

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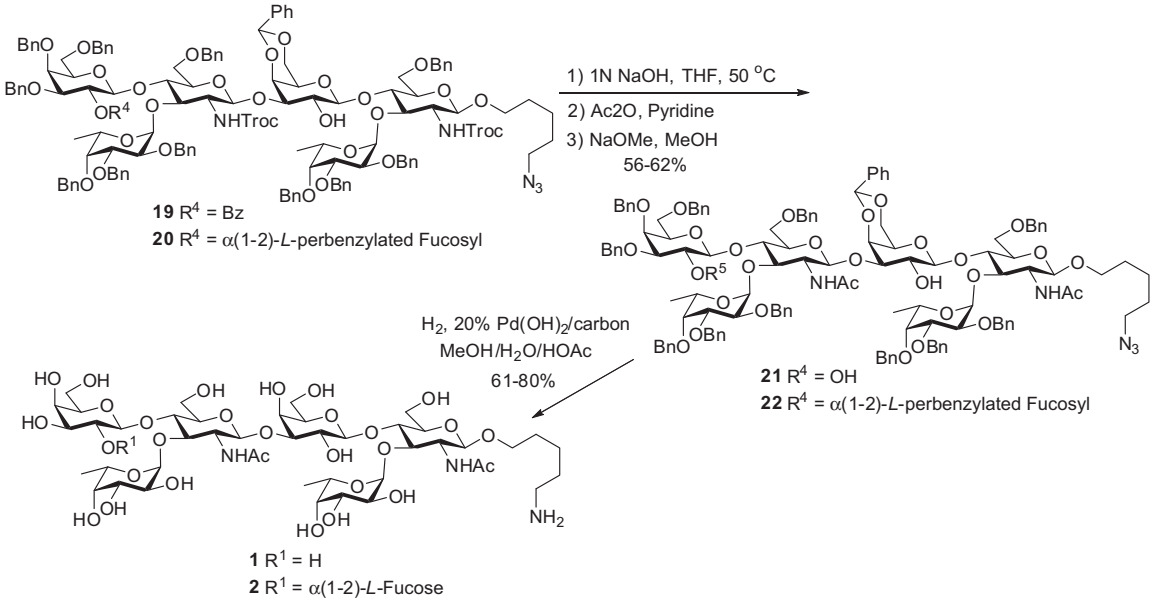

Scheme 6. Synthesis of Le^x dimer and KH-1 epitopes.

Supplementary data

Supplementary data associated with (full characterization of all new compounds (**1**–**2**, **6**, **19** and **20**) and copies of NMR spectra) this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.11.055.

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