Pergamon

Tetrahedron Letters 41 (2000) 855–858

TETRAHEDRON LETTERS

Novel carbohydrate scaffolds. Assembly of a uridine–mannose scaffold based on tunicamycin

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Received 9 September 1999; revised 19 November 1999; accepted 22 November 1999

Abstract

A disaccharide scaffold based on tunicamycin was synthesized from D-uridine and L-mannose. The key step in disaccharide assembly was a β-mannosylation performed using Crich's modification of the sulfoxide glycosylation method. The scaffold described contains two orthogonal derivatization sites and will be used in the search for novel biologically active compounds.

Keywords: carbohydrates; antibiotics; combinatorial chemistry.

As part of our drug discovery program, we are interested in the construction of scaffolds based on therapeutically interesting carbohydrates and glycoconjugates.[1] Such scaffolds can be utilized as building blocks in the assembly of combinatorial libraries, with the objective of exploring the SAR of the original drug and identifying novel biologically active agents.[2]

This paper reports work in the construction of one such scaffold based on the tunicamycins (**1**), a family of nucleoside antibiotics isolated from the fermentation broths of *Streptomyces lysosuperificus*.[3] These drugs have a general structure composed of a fatty acid chain, uracil, *N*-acetyl-D-glucosamine and an undecose sugar named tunicamine.[4] The tunicamycins have been shown to inhibit a wide variety of lipid carrier-dependent protein glycosylations, having great potential as antibiotic and antitumor agents.[5] For example, in bacteria the tunicamycins inhibit the conversion of UDP-MurNAc to lipid I catalyzed by the MraY enzyme, inhibiting cell wall biosynthesis and leading to bacterial death.[6] Unfortunately, tunicamycins have not enjoyed use as human therapeutic agents because they are also toxic to mammalian cells, interfering with dolichol–diphosphoryl–GlcNAc synthesis and inhibiting oligosaccharide biosynthesis.[7] Nevertheless, since tunicamycins inhibit enzymes with distinct substrate requirements, it is plausible that distinct features of the drug may be recognized by each affected enzyme. Tunicamycin analogs may thus have differential inhibitory effects towards eukaryotic and prokaryotic cells, allowing for the possible targeting of pathogenic cells over mammalian cells. Because of the

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0040-4039/00/$ - see front matter
PII: S0040-4039(99)02209-1

great structural complexity of the drugs, limited work besides chemical degradation has been devoted to establishing a structure–activity relationship for these compounds.[8]

The disaccharide scaffold described here (**2**, Fig. 1) has the general structure of tunicamycin, with modifications in the two-carbon bridge and the glucosamine ring. Besides simplifying the overall synthesis of **2**, these modifications may shed light on the importance of the specific groups in biological activity. Furthermore, **2** incorporates two sites (azide and hydroxyl groups) that can be derivatized orthogonally (Fig. 1). Retrosynthetic analysis of **2** showed that the key synthetic step was formation of a β-glycosidic linkage between a L-mannosyl donor and a D-uridyl acceptor. Synthesis of β-mannosides is still one of the most difficult challenges in modern carbohydrate chemistry.[9] Since Crich and Sun have recently shown that β-mannosides can be synthesized in high yield using a modification of the sulfoxide glycosylation method,[10] this glycosylation method was chosen for construction of the disaccharide core.

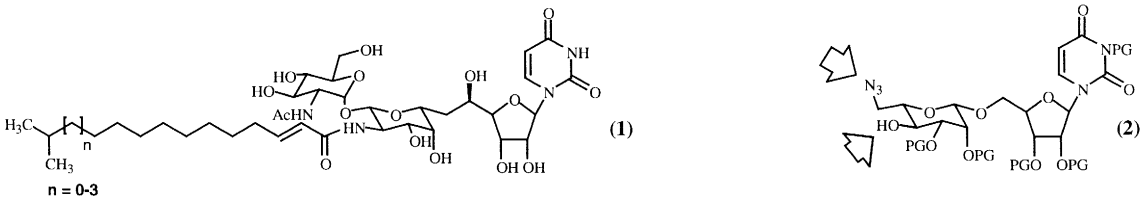

Fig. 1.

Synthesis of the D-uridyl acceptor is shown in Fig. 2. Uridine **3** was reacted with trityl chloride in pyridine in the presence of DMAP to afford the 5′-trityl derivative **4**, which was peralkylated with benzyloxymethyl chloride to generate **5**. Treatment of **5** with formic acid in acetonitrile led to clean removal of the trityl group, without loss of the BOM groups, yielding acceptor **6**.[11]

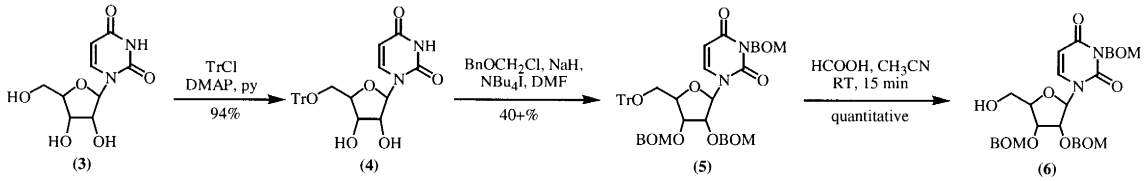

Fig. 2.

Synthesis of the L-mannosyl donor is shown in Fig. 3. L-Mannose **7** was peracetylated and reacted with PhSH and $BF_3 \cdot OEt_2$ to generate sulfides **9a**–**b**. The α-sulfide **9a** was deacetylated to the tetraol **10** and reacted with benzaldehyde dimethyl acetal to afford the 4,6-di-*O*-benzylidene compound **11**. Treatment with excess BnCl and NaH yielded the fully protected sulfide **12**, which was cleanly oxidized using mCPBA to sulfoxide **13**. Compound **13** was recrystallized as a single diastereoisomer.[12]

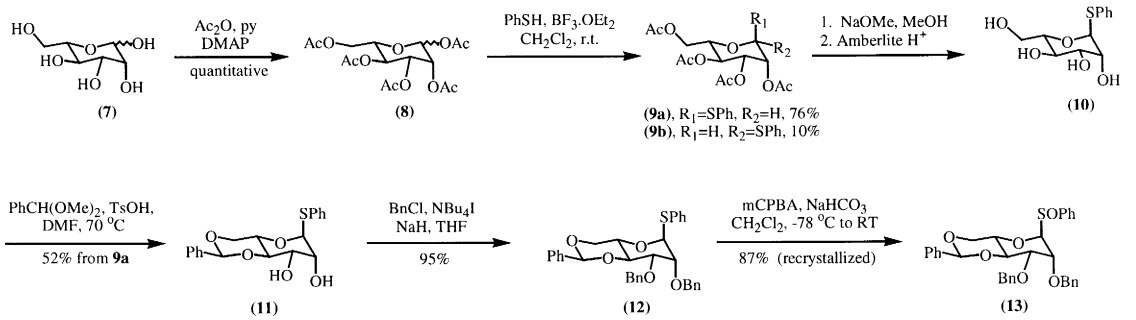

Fig. 3.

Compounds **6** and **13** were coupled using Crich's protocol for the sulfoxide glycosylation reaction (Fig. 4). A solution of **13** and base in CH_2Cl_2 was treated with Tf_2O at −78°C, and after 5 min **6** was added. The reaction afforded 53% of the desired β-mannoside **14**,[13] along with 29% of the corresponding α-mannoside and 10% of mannosyl lactols. The benzylidene group in **14** was selectively removed with aqueous HOAc at 50°C, and the 6′-hydroxyl group was tosylated to afford **16**. Finally, the tosyl group was displaced using sodium azide, affording target scaffold **17**.[14]

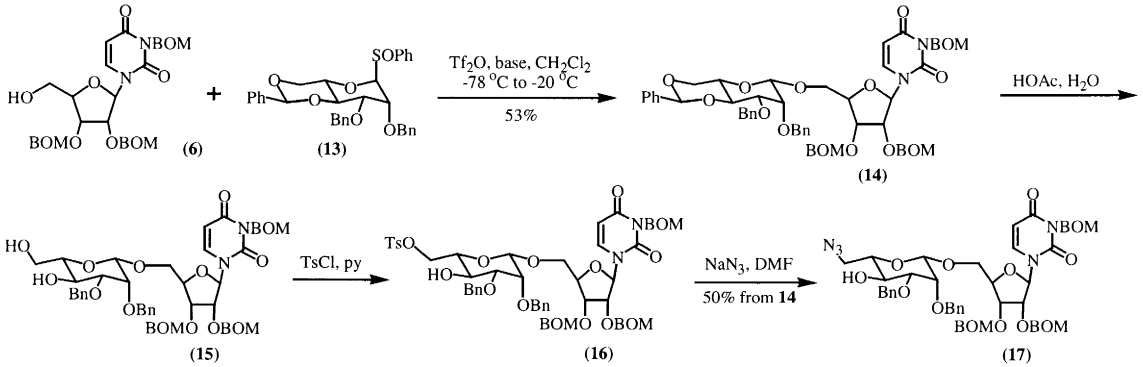

Fig. 4.

Diversity could, in principle, be introduced in **17** by selective derivatization of the 6′-azide and the 4′-hydroxyl groups on the L-mannose ring, followed by removal of protective groups (Fig. 5). In order to validate such chemical diversity strategy, **17** was treated with PMe_3 in THF–EtOH–H_2O and the resulting amine **18** was selectively acetylated using HOAc, HATU and DIPEA to afford **19**. Reaction with *n*-octadecyl isocyanate afforded the urethane **20**, which was hydrogenated to generate the expected bis-derivatized product **21**.

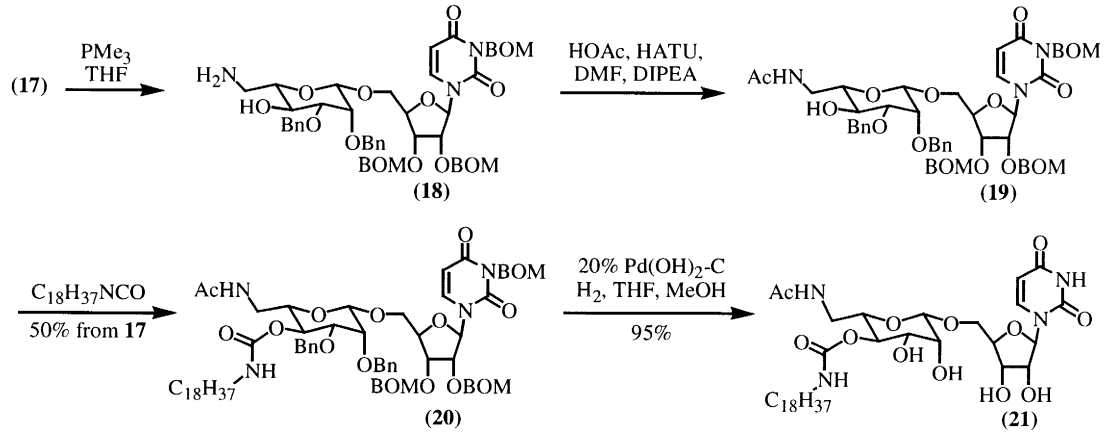

Fig. 5.

In summary, we have reported the efficient synthesis of a disaccharide scaffold **17** based on the tunicamycins. Scaffold **17** was synthesized from two commercially available building blocks (L-mannose and D-uridine) and the key step in the synthesis involved the use of Crich's modification of the sulfoxide glycosylation method to obtain a β-mannoside in good yield. Compound **17** presents a functional group dyad (the azide and the free hydroxyl groups) that can be orthogonally derivatized to generate di-substituted disaccharide scaffolds. Based on this combinatorial flexibility, we are currently pursuing the synthesis of tunicamycin analogs based on **17** and other related scaffolds. Biological evaluation of such

compounds should shed light on the mechanism of action of the tunicamycins and help refine our initial choice of scaffold structure.

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11. Analytical data for **6**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.55–7.20 (m, 20H), 5.64 (1H, s), 4.82 (1H, d, *J*=12 Hz), 4.66 (1H, d, *J*=12 Hz), 4.58 (2H, d, *J*=1.8 Hz), 4.50 (1H, br), 4.39 (1H, dd (br), *J*=1.2, 3 Hz), 4.34–4.20 (3H, m), 4.10 (1H, td, *J*=4.5, 9, 9 Hz), 3.76 (1H, t, *J*=9.9 Hz). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 141.5, 138.2, 137.3, 137.2, 131.6, 129.4, 129.0, 128.3, 128.2, 127.9, 127.8, 127.6, 126.0, 124.3, 101.6, 97.6, 78.0, 76.2, 73.5, 73.2, 72.8, 70.0, 68.2. MS (ES) *m/z* obs. 574 [M+NH_4^+].
12. Analytical data for **13**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.79 (1H, d, *J*=8.1 Hz), 7.40–7.20 (15H, m), 5.89 (1H, d, *J*=3.3 Hz), 5.66 (1H, d, *J*=8.1 Hz), 5.38 (2H, dd, J_{app}=9.9, 13.2 Hz), 4.98 (1H, d, *J*=6.9 Hz), 4.87 (1H, d, *J*=6.9 Hz), 4.83 (2H, m), 4.68–4.55 (5H, m), 4.45 (1H, t, *J*=3.3 Hz), 4.36 (1H, t, *J*=5.1 Hz), 4.22 (1H, d, *J*=6.0 Hz), 3.97 (1H, d (br), *J*=12.0 Hz), 3.74 (1H, dd (br), *J*=3.0, 12.0 Hz), 3.11 (1H, br). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 162.7, 150.8, 139.7, 137.6, 137.1, 128.4, 128.3, 128.2, 127.8, 127.6, 127.5, 127.5, 127.4, 101.5, 94.4, 94.2, 90.5, 82.8, 78.0, 73.0, 72.1, 69.9, 69.7, 60.7. MS (ES) *m/z* obs. 622 [M+NH_4^+].
13. The determination of the stereochemistry of the new glycosidic linkage in **14** was hampered by severe overlap in NMR spectra. Removal of all protective groups in **14** (H_2, Pd/C, 1 atm) afforded **22**, for which the ^{1}H-coupled ^{13}C NMR was recorded in CD_3OD (75.4 MHz). The ^{13}C resonance of the mannosyl anomeric carbon (101.7 ppm) showed J_{H-C}=159 Hz, characteristic of β-mannosides: Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293–297. The β:α selectivity of this glycosylation (1.8:1) is lower than the selectivities generally obtained with Crich's procedure (Ref. 10) and may be associated with particular stereoelectronic characteristics of the uridyl acceptor, since the mannosyl donor has been shown to afford high β/α selectivities with primary glycosyl acceptors (Ref. 10b).
14. Analytical data for **17**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.48 (1H, d, *J*=8.4 Hz), 7.4–7.2 (25H, m), 5.96 (1H, d, *J*=2.4 Hz), 5.50 (1H, d, *J*=8.1 Hz), 5.41 (1H, d, *J*=9.9 Hz), 5.36 (1H, d, *J*=9.9 Hz), 4.99 (1H, d, *J*=6.9 Hz), 4.9–4.8 (4H, m), 4.7–4.5 (9H, m), 4.4–4.3 (5H, m), 4.22 (1H, dd, *J*=2.7, 11.4 Hz), 3.9–3.8 (3H, m), 3.5–3.4 (3H, m), 3.32 (1H, dd, *J*=2.7, 9.3 Hz). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 162.3, 150.7, 138.4, 138.1, 137.8, 137.2, 128.7, 128.4, 128.3, 128.2, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 101.6, 101.4, 94.3, 89.9, 81.6, 80.8, 78.1, 76.3, 74.5, 74.2, 73.0, 72.2, 71.6, 70.2, 69.9, 69.9, 67.7, 67.5, 51.5. MS (ES) *m/z* obs. 989 [M+NH_4^+].