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Synthesis of a fluorescent arabinofuranosyl disaccharide: a probe for arabinosyltransferase activity in *Mycobacterium tuberculosis*

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Abstract—(5-*N*,*N*-Dimethylaminonaphthalene-1-sulfonamidoethyl)-5-*O*-(α-D-arabinofuranosyl)-α-D-arabinofuranoside **1** was synthesized as a fluorescent probe for the determination of arabinosyltransferase activity in *Mycobacterium tuberculosis*.

Tuberculosis remains one of the primary infectious diseases worldwide, particularly in developing nations.[1] The appearance of multiple drug-resistant forms of the bacterium, and the association with and early onset of tuberculosis in AIDS patients, has increased awareness of the disease in developed nations and the United States.[2] Public health officials are now calling for increased funding directed at understanding the tuberculosis bacillus and developing more effective vaccines and new drugs.[3] Historically, the mycobacterial cell wall has been a robust target for antitubercular agents.[4] With the availability of the genetic sequence of tuberculosis, and recent developments in the elucidation of the cell wall structure, dynamic new targets are available that offer the promise for production of highly selective antimycobacterial agents with novel mechanisms of action.[5] In particular, the mycobacterial arabinogalactan is a proven target of the active first line antitubercular agent ethambutol.[6] The elucidation of the structure of this crucial cell wall component highlights the arabinosyl- and galactosyltransferases as exciting new targets for drug intervention.[7] Both galactose and arabinose exist in the furanose form in the cell wall, sugar structures not found in humans. Since glycosyltransferases are quite specific for their sugar substrates, agents directed at the arabinogalactan should be exquisitely selective for the bacterial pathogen. Accordingly, several groups have reported work in understanding the mode of action of ethambutol, development of an arabinosyltransferase assay system, and production of disaccharide analog substrates that are a basis for probe and inhibitor development.[7–11]

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The development of new drugs targeting the mycobacterial cell wall arabinogalactan requires a robust, inexpensive assay system to facilitate drug screening. Lee et al. have reported the development of an effective assay for arabinosyltransferase activity in mycobacteria utilizing a synthetic, radiolabeled form of the mycobacterial arabinose donor [1-^{14}C]-β-D-arabinofuranosyl-1-monophosphoryldecaprenol (DPA).[12] Simply, the radiolabeled donor DPA and synthetic disaccharide acceptors are incubated with mycobacterial cell wall preparations containing the arabinosyltransferase activity. The reaction is terminated and the preparation is analyzed by thin-layer chromatography (TLC) and autoradiography of the developed plates. Bands on the developed film correspond to disaccharide analogs that have accepted a radiolabeled arabinofuranose from the DPA donor. Development of this assay represented a breakthrough in studies of this transferase system and offered new opportunities for medicinal chemists to design agents directed at this crucial pathway. This assay, however, suffers from several shortcomings including: (1) low throughput; (2) requirement for radiolabeled DPA; and (3) special handling and waste disposal issues.

Herein, we report the preparation of a fluorescently-labeled arabinofuranosyl disaccharide **1**, based on the highly fluorescent 5-*N*,*N*-dimethylaminonapthalene-1-sulfonyl (dansyl)[13] function, as an acceptor that will be useful for the development of a second-generation assay system for the mycobacterial arabinosyltransferases. A fluorescence-based assay can be very sensitive and offers a number of alternative screening formats[14] in addition to the simple TLC method described by Lee et al.[12] Ideally, one of these approaches will lead to a true

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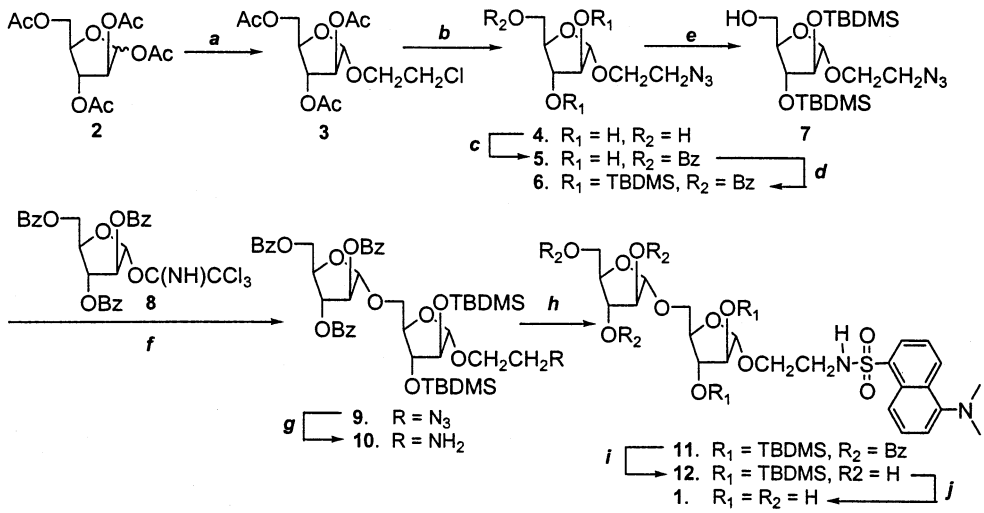

Scheme 1. Synthesis of (5-*N*,*N*-dimethylaminonaphthalene-1-sulfonamidoethyl)-5-*O*-(α-D-arabinofuranosyl)-α-D-arabinofuranoside. *Reagents and conditions*: (a) $Cl(CH_2)_2OH$, $SnCl_4$, CH_3CN, 30 min, rt, 85%; (b) NaN_3, DMF, 84°C, 6 h, 7N NH_3/MeOH, rt, overnight, 70% in two steps; (c) BzCl, pyridine, −78°C to rt, overnight, 58%; (d) TBDMSCl, DMF, imidazole, 87°C, 2 days, 81%; (e) 7N NH_3/MeOH, rt, overnight, 93%; (f) $BF_3{\cdot}Et_2O$, CH_2Cl_2, −20°C, 2 h, 66%; (g) Ph_3P, benzene, H_2O, 50°C, 6 h, 87%; (h) dansyl chloride, *N*-methylimidazole, CH_2Cl_2, 0°C, 3 h, 95%; (i) 7N NH_3/MeOH, rt, overnight, 93%; (j) $Et_4N^+F^-$, THF, rt, overnight, 97%.

high throughput assay that will allow screening of large numbers of compounds for drug development.

The synthesis of **1** is represented in Scheme 1. 1,2,3,5-Tetra-*O*-acetyl-D-arabinofuranoside **2** was readily obtained from D-arabinose by reported methods.[15] Reaction of **2** with chloroethanol in the presence of $SnCl_4$ gave **3** as the pure α-isomer after column chromatography.[10] The chloroethyl compound **3** was then heated with NaN_3 in dry DMF for 6 hours followed by deacetylation with 7N NH_3/MeOH to give **4** in an overall yield of 70% (two steps) after purification. Selective blocking of the 5-position with a benzoyl group was accomplished by adding 1.0 equiv. of benzoyl chloride in dry pyridine at −78°C followed by warming to RT and leaving overnight. Workup and chromatography gave compound **5** in 58% yield. Both the 2- and 3-positions were blocked with a TBDMS group to give compound **6** followed by treatment with 7N NH_3/MeOH to obtain the acceptor **7** in high yield. The trichloroacetimidate donor **8**[16] (1.2 equiv.) and the acceptor azidoethyl-2,3-*O*-di-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (**7**) (1.0 equiv.) were reacted for 2 h in the presence of the promoter $BF_3{\cdot}Et_2O$ (1.0 equiv., dissolved in 2 mL of dry CH_2Cl_2). Additions were done at 0°C, and the reaction was carried out under an inert atmosphere in dry CH_2Cl_2 at rt over powdered 4 Å molecular sieves. The reaction mixture was diluted with $CHCl_3$, followed by workup. Column chromatography on silica gel G (70–230 mesh) afforded the pure disaccharide **9** in 66% yield. The azido group was reduced by heating with Ph_3P in a benzene/H_2O mixture followed by rapid purification via flash chromatography to afford compound **10**. The relative instability of **10** necessitated immediate reaction with dansyl chloride in the presence of *N*-methylimidazole. The fluorescently labeled disaccharide **11** was quite stable, and was obtained in high yield. Lastly, compound **11** was deprotected via **12** to give the fluorescent target **1**. All compounds were characterized by CHN analysis, FABMS and NMR spectroscopy, and data for key compounds are given below.[17] The NOE, decoupling, D_2O exchanged and DEPT experiments were performed as required in order to confirm NMR assignments and stereochemistry at the anomeric center.

In summary, we have reported a simple and efficient synthesis of a highly fluorescent, dansylated arabinofuranosyl disaccharide for potential use in assaying arabinosyltransferase activity in *Mycobacterium tuberculosis*.

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17. Analytical data of selected targets. *Compound* ***7***: FABMS (LiCl): 454.2 $[M+Li]^+$. $C_{19}H_{41}N_3O_5Si_2$ (found: C, 50.92; H, 9.00; N, 9.58; requires: C, 50.97; H, 9.23; N, 9.39). ^1H NMR ($CDCl_3$): δ 4.85 (1H, d, $J=1.3$ Hz, H-1), 4.07 (1H, dd, $J=1.3$, 3.1 Hz, H-2), 4.05–3.99 (2H, m, H-3, H-4), 3.89 (1H, m, OCH_2), 3.83 (1H, ddd, $J=2.3$, 4.6, 11.9 Hz, H-5_a), 3.66 (1H, ddd, $J=3.4$, 7.4, 11.9 Hz, H-5_b), 3.57 (1H, m, OCH_2), 3.45 (1H, m, CH_2), 3.34 (1H, m, CH_2), 0.90, 0.89, 0.88, 0.11, 0.09, 0.08 (CH_3). ^{13}C NMR ($CDCl_3$): δ 108.54 (C-1), 84.49 (C-4), 83.72 (C-2), 78.23 (C-3), 66.30 (OCH_2), 61.63 (C-5), 50.69 (CH_2), 25.71, 25.62 (CH_3), 17.81, 17.75 (C), −4.42, −4.76, −4.80, −4.98 (CH_3). *Compound* ***9***: FABMS (LiCl): 898.5 $[M+Li]^+$. $C_{45}H_{61}N_3O_{12}Si_2\cdot 1.7H_2O$ (found: C, 58.45; H, 6.66; N, 4.57; requires: C, 58.57; H, 6.85; N, 4.55). ^1H NMR ($CDCl_3$): δ 8.09–7.98, 7.62–7.24 (m, aromatic), 5.59 (1H, d, $J=1.1$ Hz, H-2′), 5.56 (1H, d, $J=4.8$ Hz, H-3′), 5.38 (1H, s, H-1′), 4.84 (1H, dd, $J=3.2$, 11.8 Hz, H-$5'_a$), 4.83 (1H, d, $J=1.4$ Hz, H-1), 4.69 (1H, dd, $J=4.7$, 11.8 Hz, H-$5'_b$), 4.62 (1H, ddd, $J=3.2$, 4.7, 4.7 Hz, H-4′), 4.08 (1H, m, H-4), 4.06 (1H, dd, $J=1.4$, 3.7 Hz, H-2), 3.99 (1H, dd, $J=3.7$, 6.5 Hz, H-3), 3.89 (1H, dd, $J=5.2$, 11.2 Hz, H-5_a), 3.82 (1H, m, OCH_2), 3.75 (1H, dd, $J=3.7$, 11.2 Hz, H-5_b), 3.51 (1H, m, OCH_2), 3.39 (1H, m, CH_2), 3.26 (1H, m, CH_2), 0.89, 0.87, 0.10, 0.09, 0.08, 0.07 (CH_3). ^{13}C NMR ($CDCl_3$): δ 166.18, 165.79, 165.27 (C=O), 133.48, 133.44, 133.00, 129.94, 129.85, 129.74, 129.14, 129.10, 128.49, 128.44, 128.27 (aromatic), 108.50 (C-1), 105.95 (C-1′), 84.12 (C-4), 82.09 (C-2, C-2′), 81.25 (C-4′), 79.07 (C-3), 78.05 (C-3′), 66.55 (C-5), 66.36 (OCH_2), 63.74 (C-5′), 50.75 (CH_2), 25.82, 25.69 (CH_3), 17.87, 17.81 (C), −4.27, −4.64, −4.67, −4.90 (CH_3). *Compound* ***10***: FABMS (LiCl): 872.7 $[M+Li]^+$. $C_{45}H_{63}NO_{12}Si_2$ (found: C, 62.14; H, 7.03; N, 1.52; requires: C, 62.40; H, 7.33; N, 1.62). ^1H NMR ($CDCl_3$): δ 8.09–7.98, 7.71–7.39 (m, aromatic), 5.58 (1H, s, H-2′), 5.57 (1H, d, $J=7.0$ Hz, H-3′), 5.37 (1H, s, H-1′), 4.84 (1H, dd, $J=3.1$, 11.8 Hz, H-$5'_a$), 4.83 (1H, d, $J=1.5$ Hz, H-1), 4.69 (1H, dd, $J=4.7$, 11.8 Hz, H-$5'_b$), 4.62 (1H, ddd, $J=3.1$, 4.7, 7.0 Hz, H-4′), 4.07 (1H, m, H-4), 4.04 (1H, dd, $J=1.5$, 3.4 Hz, H-2), 3.99 (1H, dd, $J=3.4$, 5.8 Hz, H-3), 3.89 (1H, dd, $J=5.4$, 11.0 Hz, H-5_a), 3.72 (1H, dd, $J=4.3$, 11.0 Hz, H-5_b), 3.68 (1H, m, OCH_2), 3.40 (1H, m, OCH_2), 2.82 (2H, m, CH_2), 1.95 (2H, br s, NH_2), 0.88, 0.87, 0.10, 0.09, 0.08, 0.07 (CH_3). ^{13}C NMR ($CDCl_3$): δ 166.20, 165.81, 165.30 (C=O), 133.49, 133.45, 133.00, 132.14, 132.02, 131.93, 131.90, 129.14, 129.08, 128.56, 128.40 (aromatic), 108.27 (C-1), 105.92 (C-1′), 83.84 (C-4), 82.41 (C-2), 82.12 (C-2′), 81.18 (C-4′), 79.18 (C-3), 78.02 (C-3′), 69.53 (OCH_2), 66.85 (C-5), 63.73 (C-5′), 41.96 (CH_2), 25.80, 25.70 (CH_3), 17.88, 17.82 (C), −4.33, −4.54, −4.66, −4.78 (CH_3). *Compound* ***11***: FABMS (LiCl): 1104.7 $[M+Li]^+$. $C_{57}H_{74}N_2O_{14}SSi_2\cdot 2H_2O$ (found: C, 60.01; H, 6.80; N, 2.29; requires: C, 60.29; H, 6.92; N, 2.47). ^1H NMR ($CDCl_3$): δ 8.51 (1H, d, $J=8.6$ Hz, aromatic), 8.30 (1H, d, $J=8.7$ Hz, aromatic), 8.22 (1H, dd, $J=1.2$, 7.3 Hz, aromatic), 8.06–7.96, 7.60–7.24 (m, aromatic), 7.14 (1H, d, $J=7.6$ Hz, aromatic), 5.57 (1H, s, H-2′), 5.56 (1H, d, $J=4.0$ Hz, H-3′), 5.32 (1H, s, H-1′), 5.30 (1H, m, NH), 4.83 (1H, dd, $J=3.1$, 11.6 Hz, H-$5'_a$), 4.69 (1H, d, $J=1.8$ Hz, H-1), 4.68 (1H, dd, $J=4.8$, 11.6 Hz, H-$5'_b$), 4.62 (1H, ddd, $J=3.1$, 4.0, 4.8 Hz, H-4′), 3.98 (2H, m, H-3, H-4), 4.04 (1H, dd, $J=1.8$, 3.3 Hz, H-2), 3.84 (1H, dd, $J=4.6$, 10.9 Hz, H-5_a), 3.61 (1H, dd, $J=3.5$, 10.9 Hz, H-5_b), 3.56 (1H, m, OCH_2), 3.40 (1H, m, OCH_2), 3.05 (2H, m, CH_2), 2.86 (2H, s, 2×N-CH_3), 0.86, 0.85, 0.08, 0.07, 0.06, 0.05 (CH_3). ^{13}C NMR ($CDCl_3$): δ 166.14, 165.78, 165.26

(C=O), 151.90, 134.86, 133.43, 133.40, 132.97, 130.29, 129.87, 129.81, 129.70, 129.62, 129.38, 129.02, 128.44, 128.38, 128.24, 128.20, 123.10, 118.93, 115.14 (aromatic), 108.33 (C-1), 106.08 (C-1′), 83.48 (C-4), 82.60 (C-2), 82.12 (C-2′), 81.14 (C-4′), 78.62 (C-3), 77.95 (C-3′), 66.65 (C-5), 66.35 (OCH_2), 63.66 (C-5′), 45.35 (N-CH_3), 43.28 (CH_2), 26.71 (CH_3), 17.82 (C), −4.36, −4.61, −4.69, −4.81 (CH_3). *Compound* ***1***: FABMS (LiCl): 565.0 $[M+Li]^+$. $C_{24}H_{34}N_2O_{11}S\cdot 1.8H_2O$ (found: C, 48.79; H, 6.35; N, 4.57; requires: C, 48.77; H, 6.41; N, 4.74). ^{1}H NMR ($CDCl_3$): δ 8.53 (1H, d, $J=8.6$ Hz, aromatic), 8.29 (1H, d, $J=8.7$ Hz, aromatic), 8.22 (1H, dd, $J=1.2$, 7.4 Hz, aromatic), 7.58–7.49 (m, aromatic), 7.18 (1H, d, $J=7.6$ Hz, aromatic), 5.30 (1H, m, NH, D_2O exchange), 5.07 (1H, s, H-1′), 4.68 (1H, d, $J=1.5$ Hz, H-1), 4.17 (1H, dd, $J=2.3$, 4.5 Hz, H-4′), 4.05 (1H, d, $J=0.8$ Hz, H-2′), 3.99 (3H, m, H-4, H-3′, 2′-OH), 3.92 (1H, dd, $J=1.7$, 3.4 Hz, H-2), 3.86 (1H, dd, $J=2.5$, 11.8 Hz, H-5′$_a$), 3.77 (3H, m, H-3, H-5a, H-5′$_b$), 3.58 (1H, dd, $J=3.2$, 10.7 Hz, H-5$_b$), 3.50 (2H, m, OCH_2), 3.25 (1H, d, $J=11.4$ Hz, 3′-OH), 3.08 (2H, m, CH_2), 2.89 (2H, s, 2×N-CH_3), 0.87, 0.86, 0.08, 0.06, 0.05 (CH_3). ^{13}C NMR ($CDCl_3$): δ 151.11, 134.63, 130.21, 129.44, 129.22, 129.19, 128.80, 123.89, 119.19, 115.82 (aromatic), 107.71 (C-1), 107.59 (C-1′), 84.25 (C-4′), 82.54 (C-2), 81.19 (C-4), 80.98 (C-2′), 76.91 (C-3), 76.83 (C-3′), 66.73 (C-5′), 66.30 (OCH_2), 61.47 (C-5), 45.02 (N-CH_3), 42.17 (CH_2).