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# Synthesis of an Arabinofuranosyl Disaccharide Photoaffinity Probe for Arabinosyltransferase Activity in *Mycobacterium tuberculosis*

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**Abstract**—(5-Azidonaphthalene-1-sulfonamidoethyl)-5-*O*-(α–arabinofuranosyl)-α-D-arabinofuranoside **1** was synthesized as a photoaffinity probe for the determination of arabinosyl transferase activity and for the identification of binding and functional sites in *Mycobacterium tuberculosis*.


*Mycobacterium tuberculosis* (Mtb), an intracellular pathogen, continues to be a primary cause of morbidity and mortality worldwide; it is currently estimated that one-third of the world's population is infected with the bacillus that causes tuberculosis.[1] The emergence of bacterial resistance to existing antitubercular agents has become a significant concern for the continued, effective treatment of tuberculosis, and this problem has necessitated a renewed search for novel antimycobacterial drugs.[1c] The continuing development of tuberculosis as an opportunistic pathogen in AIDS patients, even with the advent of HAART,[2] also highlights the need for new antitubercular agents and improved treatment regimens. The structural and functional integrity of the cell wall of Mtb is essential for growth and survival of the bacillus within macrophages of the infected host, and this infrastructure has traditionally been an excellent target for drug treatment.[3] With the thorough elucidation of the mycobacterial cell wall structure and the publication of the genome of Mtb,[4] a number of new targets have been identified in the cell wall that offer the hope of improved therapies.[5]

Ethambutol (EMB) is a frontline anti-tubercular drug that targets the bacterial cell wall.[6,7] It has been proposed that EMB directly targets the Emb proteins (EmbA-C) causing alterations in the synthesis of the arabinan portion of the mycobacterial cell wall arabinogalactan (AG) and the lipoarabinomannan (LAM); the Emb proteins are hypothesized to contain an arabinosyltransferase activity required for addition of arabinose units into the AG and LAM components of the cell wall.[6,7] A significant body of work has been reported relating to the mode of action of ethambutol, development of an arabinosyltransferase assay system, and production of disaccharide analogue substrates that are a basis for probe and inhibitor development.[8–11]

To date, the evidence that ethambutol targets a putative arabinosyltransferase activity through the Emb proteins is indirect, and is based on resistance and gene knockout studies.[6,7] Utilizing a similar synthetic approach as reported for a fluorescent disaccharide probe,[11] herein we report the preparation of a photoactivatable arabinofuranosyl disaccharide **1** that can potentially be used to fluorescently label proteins that utilize this disaccharide as a substrate for arabinosyl transfer reactions (e.g., the arabinosyltransferases). Our specific goal is to ascertain whether these fluorescent disaccharides will label the proposed arabinosyltransferase proteins (EmbA-C), but this general approach may be applicable to other glycosyltransferases.

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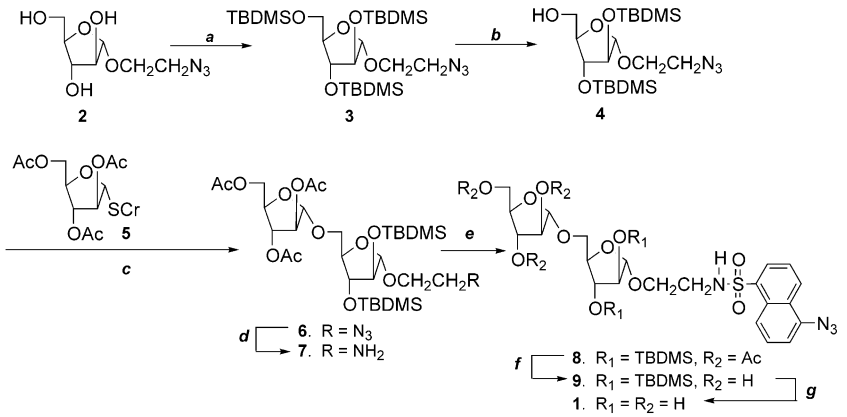


**Scheme 1.** Synthesis of (5-azidonaphthalene-1-sulfonamidoethyl)-5-*O*-(α-D-arabinofuranosyl)-α-D-arabinofuranoside. Reagents and conditions: (a) TBDMSCl, DMF, imidazole, 60 °C, 2 days, 90%; (b) TFA–water (1:1), 0 °C, 68%; (c) NIS, $Sn(OTf)_2$, $CH_2Cl_2$, −20 °C, 2 h, 91%; (d) $HCO_2NH_4$, MeOH, 5% Pd/C, rt, 2 h, 87%; (e) 5-azidonaphthalene sulfonyl chloride, *N*-methylimidazole, $CH_2Cl_2$, 0 °C, 3 h, 81%; (f) 7 N $NH_3$/MeOH, rt, overnight, 87%; (g) $Et_4N^+F^-$, THF, rt, overnight, 80%.

Photoaffinity labeling is a useful tool in the identification of binding proteins for various ligands, as well as for locating enzyme functional sites.[12] This technique requires an aromatic azide conjugated to an affinity ligand that on protein binding and exposure to UV light photodecomposes and generates a reactive nitrene intermediate that can form a cross-link between the ligand and its protein binding partner. Herein, we have used 5-azidonapthalene-1-sulfonyl chloride,[13] as a heterobifunctional reagent (both a photocoupling moiety as well as an end-product fluorescent label) that was successfully coupled with the Ara*f*(α 1,5)Ara*f* disaccharide.

The synthesis of the target probe **1** is represented in Scheme 1. Ethylazido-α-D-arabinofuranoside (**2**) was prepared starting from 1,2,3,5-tetra-*O*-acetyl-D-arabinofuranoside that was readily obtained from D-arabinose by reported methods[14] and subsequent reactions with chloroethanol in the presence of $SnCl_4$[8a] followed by the reaction with $NaN_3$ in dry DMF for 6 h at 50 °C and deacetylation with 7 N $NH_3$/MeOH. As reported earlier, **4** has been prepared through the selective protection of the 5-position of **2** with a benzoyl group by adding 1.0 equiv of benzoyl chloride in dry pyridine at −78 °C and leaving overnight at ambient temperature, followed by protection of the 2- and 3-OH groups with TBDMSCl, and removal of the 5-benzoyl group.[11] In the present case, however, we have adopted a shorter, more efficient route for the synthesis of **4**. **2**[11] was first reacted with TBDMSCl in presence of imidazole at 60 °C for 2 days affording **3** in 90% yield, followed by a reported procedure[15] for the selective deprotection of the 5-OTBDMS group in the presence of other TBDMS-blocked hydroxyl groups using TFA–water (1:1) at 0 °C. The preparation of **4** from **3** was successfully achieved in one step with a yield of 68% after column purification by the above method. The 1-thiocresyl-2,3,5-tri-*O*-acetyl-α-D-arabinofuranoside donor **5**[10a] (1.2 equiv) and the acceptor azidoethyl-2,3-*O*-di-*tert*-butyldimethylsilyl-α-D-arabinofuranoside **4** (1.0 equiv) were reacted for 2 h in the presence of the promoter $Sn(OTf)_2$ (0.1 equiv) and *N*-iodosuccinimide (1.2 equiv). Additions of the reagents, and the subsequent reaction, were carried out at −20 °C under an argon atmosphere in dry $CH_2Cl_2$ over powdered 4 Å molecular sieves. The reaction mixture was diluted with $CHCl_3$, followed by a standard workup. Column chromatography on Silica gel G (70–230 mesh) afforded the pure disaccharide **6** in 91% yield. The azido group was reduced by reaction with ammonium formate in methanol using 5% Pd/C as catalyst for 2 h at room temperature followed by rapid purification via flash chromatography to afford compound **7** in 87% yield. The relative instability (probably a result of the acetyl blocking groups) of **7** necessitated immediate reaction with 5-azidonaphthalene sulfonyl chloride[13] in the presence of *N*-methylimidazole at 0 °C for 4 h. The disaccharide **8** was quite stable, and was obtained in high yield (81%). Lastly, **8** was deprotected via **9** to give the final target compound (5-azidonaphthalene-1-sulfonamidoethyl-5-*O*-(α-D-arabinofuranosyl)-α-D-arabinofuranoside **1**.

All compounds were characterized by CHN analysis, FABMS, and NMR spectroscopy, and data for key compounds are given below.[16] 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 of sugars.

Compound **1** (SRI 20477) was assayed for arabinosyltransferase activity at a range of concentrations (0.5–8.0 mM) using an established assay format.[17] Based on previous use of specific arabinose neoglycolipid acceptors,[17] **1** was synthesized and corresponds to the major structural motif found within the arabinan of the mycobacterial arabinogalactan. Assays performed in the presence of membranes resulted in excellent [$^{14}$C]Ara*f* incorporation from DP-[$^{14}$C]A for **1** (see Fig. 1). A concentration of approximately 8 mM for **1** resulted in maximum arabinosyltransferase activity. The TLC/autoradio-graphy (Fig. 1B) demonstrated the enzymatic conversion of **1** to its corresponding trisaccharide product, [$^{14}$C]Ara*f* to the 5′-OH and 2′-OH of **1** as reported for the previously characterized arabinose neoglycolipid acceptors.[17] Calculation of kinetic constants (Fig. 1A) revealed that **1** possessed a $K_m$ value

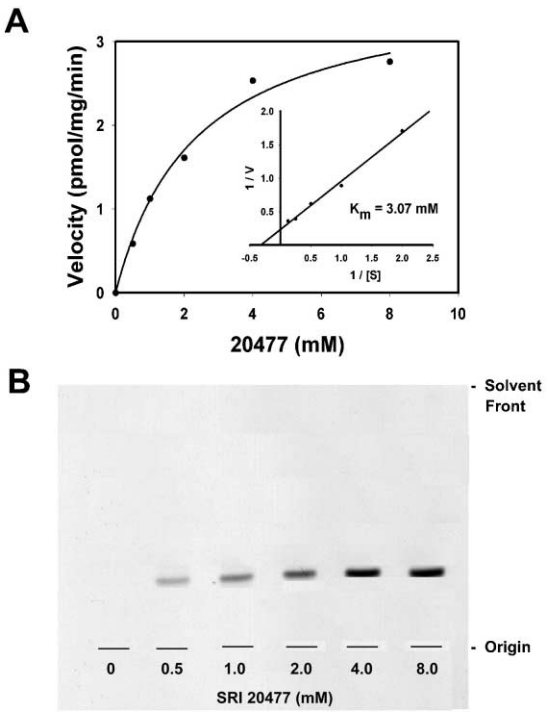


**Figure 1.** Analysis of acceptor **1** (SRI 20477). (A) Kinetic analysis of acceptor **1**. The inset illustrates the double reciprocal plot for **1** as a substrate for the mycobacterial arabinosyltransferase. (B) An autoradiogram of reactions products produced through the inclusion of **1**, mycobacterial membranes and DP[$^{14}$C]A. Lane 1, no acceptor; lane 2, 0.5 mM; lane 3, 0.1 mM; lane 4, 2.0 mM; lane 5, 4.0 mM; and lane 6, 8.0 mM. TLC/autoradiography was performed using chloroform/methanol/ammonium hydroxide/water (65:25:0.4:3.6) and products revealed through exposure to Kodak X-Omat film at −70 °C for 3 days.

of 3.07 mM and $V_{max}$ is 4.26 pmol/mg of protein/min, respectively.

In summary, we have reported a simple and efficient synthesis, and the corresponding acceptor activity, of a photoactivatable arabinofuranosyl disaccharide for potential use in determining the identification of the arabinosyltransferase activity of MTB that utilizes a 1,5-linked arabinofuranose containing disaccharide. Such compounds have potential utility for the direct identification of glycosyltransferases as well as localization of protein active sites after protein sequencing. Photoaffinity labeling experiments are in progress using established protocols,[13] and results will be published in due course.

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16. Analytical data of selected targets. Compound **3**: ESI-MS 584 $[M+Na]^+$. $^1H$ NMR (300 MHz, $CDCl_3$) δ 4.80 (1H, d, $J_{1,2}=1.5$ Hz, H-1), 4.04 (1H, dd, $J_{1,2}=1.5$ Hz, $J_{2,3}=3.2$ Hz, H-2), 3.98 (1H, dd, $J_{2,3}=3.2$ Hz, $J_{3,4}=5.6$ Hz, H-3), 3.93 (1H, ddd, $J_{3,4}=5.6$ Hz, $J_{4,5a}=4.3$ Hz, $J_{4,5b}=4.8$ Hz, H-4), 3.74 (1H, dd, $J_{4,5a}=4.3$ Hz, $J_{5a,5b}=11.1$ Hz, H-$5_a$), 3.68 (1H, dd, $J_{4,5b}=4.8$ Hz, $J_{5a,5b}=11.1$ Hz, H-$5_a$), 3.56 (1H, m, $OCH_2$), 3.44 (1H, m, $CH_2N_3$), 3.32 (1H, m, $CH_2N_3$), 4.02 (1H, m, $OCH_2$), 0.90, 0.89, 0.88 (each 9H, s, 9×$CH_3$), 0.09, 0.08, 0.069, 0.065 (s, 6×$CH_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 108.84 (C-1), 84.84 (C-4), 84.14 (C-2), 78.61 (C-3), 66.29 ($OCH_2$), 62.85 (C-5), 50.86 ($CH_2N_3$), 25.94, 25.80, 25.74 ($CH_3$), 18.40, 17.89, 17.87 (3×C),-4.65,-4.69,-4.87 (6×$CH_3$). Compound **8**: ESI-MS 911 $[M+H]^+$. $C_{40}H_{62}N_4O_{14}SSi_2$. 1.0$H_2O$ (Found: C, 51.68; H, 6.91; N, 6.11. requires C, 51.70; H, 6.94; N, 6.03). $^1H$ NMR (300 MHz, $CDCl_3$) δ 8.47 (1H, dd, $J=0.8$, 8.0 Hz, aromatic), 8.40 (1H, ddd, $J=0.9$, 1.1, 8.5 Hz, aromatic), 8.29 (1H, dd, $J=1.2$, 7.3 Hz, aromatic), 7.66 (1H, dd, $J=7.6$, 8.6 Hz, aromatic), 7.55 (1H, dd, $J=7.5$, 8.6 Hz, aromatic), 7.37 (1H, dd, $J=0.8$, 7.6 Hz, aromatic), 5.43 (1H, m, NH), 5.14 (1H, d, $J_{2',3'}=1.5$ Hz, H-2′), 5.07 (1H, s, H-1′), 4.99 (1H, dd, $J_{2',3'}=1.5$ Hz, $J_{3',4'}=4.9$ Hz, H-3′), 4.66 (1H, d, $J_{1,2}=1.9$ Hz, H-1), 4.43 (1H, dd, $J_{4',5'a}=3.1$ Hz, $J_{5'a,5'b}=11.4$ Hz, H-$5'_a$), 4.29 (1H, ddd, $J_{3',4'}=4.9$ Hz, $J_{4',5'a}=3.1$ Hz, $J_{4',5'b}=5.6$ Hz, H-4′), 4.23 (1H, dd, $J_{4',5'b}=5.6$ Hz, $J_{5'a,5'b}=11.4$ Hz, H-$5_b'$), 3.91 (3H, m, H-2, H-3, H-4), 3.78 (1H, dd, $J_{4,5a}=4.5$ Hz, $J_{5a,5b}=10.8$ Hz, H-$5_a$), 3.51 (3H, m, H-$5_b$, $OCH_2$), 3.10 (2H, m, $CH_2NH$), 2.10, 2.09, 2.07 (each 3H, s, OAc), 0.89, 0.86, 0.095, 0.087, 0.05 ($CH_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 170.56, 170.11, 169.53 (C=O), 137.61 (C), 135.00 (C), 130.28 (CH), 128.43, 128.00 (CH), 127.22 (C), 124.32, 121.21, 114.74 (CH), 108.44 (C-1), 106.02 (C-1′), 83.45 (C-4), 82.58 (C-2), 81.17 (C-2′), 80.50 (C-4′), 78.56 (C-3), 77.09 (C-3′), 66.79 ($OCH_2$), 66.55 (C-5), 63.28 (C-5′), 43.44 ($CH_2NH$), 25.73 (6×$CH_3$), 20.77, 20.72 (3×OAc), 17.86, 17.82 (2×C), −4.38, −4.62, −4.69, −4.83 (4×$CH_3$). Compound **9**: ESI-MS 785 $[M+H]^+$. $C_{34}H_{56}N_4O_{11}SSi_2$. 0.7$H_2O$ (Found: C, 51.18; H, 7.00; N, 6.84. requires C, 51.20; H, 7.25; N, 7.02). $^1H$ NMR (600 MHz, $CD_3OD$) δ 8.50 (1H, ddd, $J=0.6$, 1.0, 8.7 Hz, aromatic), 8.40 (1H, ddd, $J=0.6$, 1.2, 8.4 Hz, aromatic), 8.27 (1H, dd, $J=1.2$, 7.4 Hz, aromatic), 7.71 (1H, dd, $J=7.5$, 8.7 Hz, aromatic), 7.61 (1H, dd, $J=7.4$, 8.4 Hz, aromatic), 7.47 (1H, dd, $J=1.0$, 7.5 Hz, aromatic), 4.87 (1H, d, $J_{1',2'}=1.8$ Hz, H-1′), 4.58 (1H, d, $J_{1,2}=1.8$ Hz, H-1), 3.99 (1H, dd, $J_{1',2'}=1.8$ Hz, $J_{2',3'}=4.0$ Hz, H-2′), 3.94 (1H, ddd, $J_{3'4'}=6.6$ Hz, $J_{4',5'a}=3.2$ Hz, $J_{4',5'b}=4.9$ Hz, H-4′), 3.93 (1H, dd, $J_{2,3}=4.1$ Hz, $J_{3,4}=6.3$ Hz, H-3), 3.86 (1H, ddd, $J_{1',3'}=0.3$ Hz, $J_{2',3'}=4.0$ Hz, $J_{3',4'}=6.6$ Hz, H-3′), 3.80 (1H, dd, $J_{1,2}=1.8$ Hz, $J_{2,3}=4.1$ Hz, H-2), 3.74 (1H, dd, $J_{4',5'a}=3.2$ Hz, $J_{5'a,5'b}=11.9$ Hz, H-$5'_a$), 3.71 (2H, m, H-4, H-5a), 3.63 (1H, dd, $J_{4',5'b}=4.9$ Hz, $J_{5'a,5'b}=11.9$ Hz, H-$5'_b$), 3.62 (2H, m, H-$5_b$, $OCH_2$), 3.32 (1H, m, $OCH_2$), 3.08 (2H, m, $CH_2NH$), 0.88, 0.85, 0.09, 0.08, 0.03, −0.03 ($CH_3$); $^{13}C$ NMR (75 MHz, $CD_3OD$) δ 138.77 (C), 137.14 (C), 131.10 (CH), 130.52 (C), 129.15, 129.09 (CH), 128.42 (C), 125.59, 122.68, 116.07 (CH), 109.77 (C-1′), 109.64 (C-1), 85.32 (C-2, C-4′), 83.44 (C-2′), 83.24 (C-4), 79.90 (C-3), 78.45 (C-3′), 67.66 ($OCH_2$), 67.44 (C-5), 62.71 (C-5′), 44.04 ($CH_2NH$), 26.34 (2×C), 18.76, 18.73 (6×$CH_3$), −3.92, −4.25, −4.55 (4×$CH_3$). Compound **1**: ESI-MS 557 $[M+H]^+$. $C_{22}H_{28}N_4O_{11}S$. 0.5$H_2O$ (Found: C, 46.74; H, 5.01; N, 9.56. requires C, 46.72; H, 5.17; N, 9.91). $^1H$ NMR (600 MHz, $CD_3OD$) δ, 8.50 (1H, dd, $J=1.2$, 7.4 Hz, aromatic), 8.40 (1H, ddd, $J=1.1$, 1.2, 8.4 Hz, aromatic), 8.26 (1H, dd, $J=1.2$, 7.4 Hz, aromatic), 7.71 (1H, dd, $J=7.4$, 8.7 Hz, aromatic), 7.60 (1H, dd, $J=7.4$, 8.4 Hz, aromatic), 7.48 (1H, dd, $J=0.8$, 7.4 Hz, aromatic), 4.89 (1H, d, $J_{1',2'}=1.3$ Hz, H-1′), 4.63 (1H, d, $J_{1,2}=1.2$ Hz, H-1), 3.964 (1H, dd, $J_{1',2'}=1.3$ Hz, $J_{2',3'}=3.5$ Hz, H-2′), 3.961 (1H, ddd, $J_{3,4}=5.8$ Hz, $J_{4,5a}=3.3$ Hz, $J_{4,5b}=5.5$ Hz, H-4), 3.86–3.82 (3H, m, H-3, H-3′, H-4′), 3.79 (1H, dd, $J_{1,2}=1.2$ Hz, $J_{2,3}=2.8$ Hz, H-2), 3.76 (1H, dd, $J_{4',5'a}=4.8$ Hz, $J_{5'a,5'b}=10.9$ Hz, H-$5_a'$), 3.73 (1H, dd, $J_{4,5a}=3.3$ Hz, $J_{5a,5b}=11.9$ Hz, H-5a), 3.63 (1H, dd, $J_{4,5b}=5.5$ Hz, $J_{5a,5b}=11.9$ Hz, H-5b), 3.52 (1H, dd, $J_{4',5'b}=3.8$ Hz, $J_{5'a,5'b}=10.9$ Hz, H-$5_b'$), 3.51 (1H, m, $OCH_2$), 3.27 (1H, m, $OCH_2$), 3.08 (2H, m, $CH_2NH$); $^{13}C$ NMR (75 MHz, $CD_3OD$) δ 138.81 (C), 137.22 (C), 130.98 (CH), 130.47 (C), 129.11 (2×CH), 128.40 (C), 125.57, 122.63, 116.11 (CH), 109.63 (C-1′), 109.59 (C-1), 85.95 (C-4), 84.34 (C-4′), 83.06 (C-2, C-2′), 79.01 (C-3), 78.72 (C-3′), 68.02 ($OCH_2$), 67.48 (C-5), 63.08 (C-5′), 43.94 ($CH_2NH$).
17. Lee, R. E.; Brennan, P. J.; Besra, G. S. *Glycobiology* **1997**, *7*, 1121.