Supporting Information:

Noninvasive Urine Biomarker Lateral Flow Immunoassay for Monitoring Active Onchocerciasis

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Figure S1: The protein conjugated structures of **SQ1** and **DG1**.

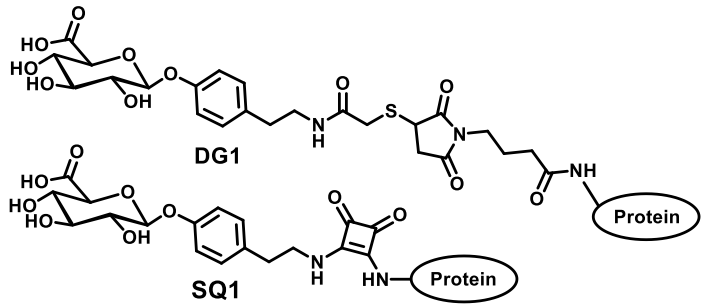


Figure S2: Synthesis of *N*-acetyl-tyramine-O-glucuronide (NATOG).

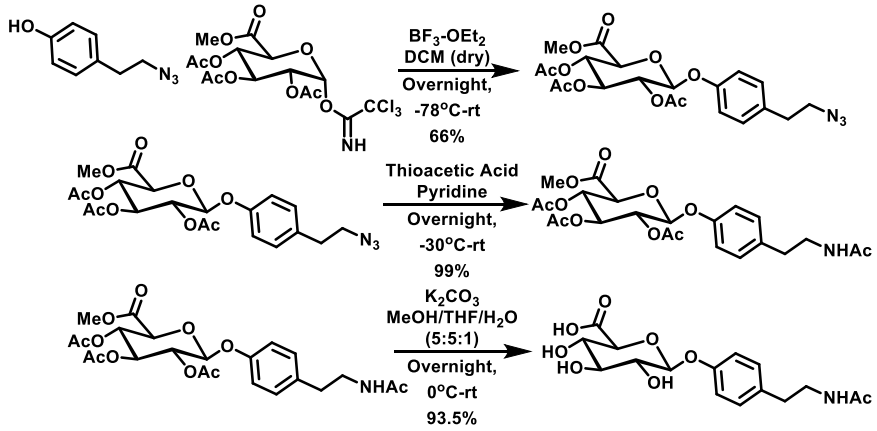


Table S1. Monoclonal antibody competition ELISA results with *N*-acetyl-tyramine-O-glucuronide (NATOG), structural analogs, and urine metabolites.

		K_{dapp} constant		
	Compound	mAb 13F9	mAb 8C7	mAb 15A3
1	NATOG	.025 µM	6.8 µM	33.0 µM
2	*N*-Acetyltyramine	> 1mM	> 1mM	> 1mM
3	D-Glucose	> 1mM	> 1mM	> 1mM
4	Glucuronic acid	> 1mM	> 1mM	> 1mM
5	*p*-Acetamidophenyl D-glucuronide	43.7 µM	> 1mM	> 1mM
6	*β*-Estradiol 3-(*β*-D-glucuronide)	n.d.	n.d.	n.d.
7	Estrone 3-(*β*-D-glucuronide)	> 1mM	> 1mM	> 1mM
8	8-Hydroxyquinoline glucuronide	> 1mM	> 1mM	> 1mM
9	Phenyl glucuronide	54.5 µM	> 1mM	> 1mM
10	*p*-Nitrophenyl glucuronide	6.0 µM	8.2 µM	> 1mM
11	L-Tyramine	> 1mM	> 1mM	> 1mM
12	L-Tyrosine	> 1mM	> 1mM	> 1mM
13	Indoxyl *β*-D-glucuronide	28..0 µM	> 1mM	> 1mM
14	D-Galactose	> 1mM	> 1mM	> 1mM
15	L-Phenylalanine	> 1mM	> 1mM	> 1mM
16	Creatinine	> 10 mM	> 10 mM	> 10 mM

Table S2. **DG1** vs **SQ1** NATOG-BSA hapten competition ELISA results showing similar binding with NATOG as the competitor.

	K_{dapp} (μM)		
Hapten	mAb 13F9	mAb 8C7	mAb 15A3
DG1	.025	6.8	33.0
SQ1	.007	4.8	27.0

Table S3. Positive and negative patient NATOG concentration values were determined by the semiquantitative lateral flow immunoassay (LFIA). Based on the cutoff (25 μM), individuals were labeled as positive or negative utilizing mass spectroscopy determined NATOG concentrations. Correct LFIA diagnoses (black) were defined as those who agreed with the mass spectroscopy determinations and incorrect diagnoses (red) were those that conflicted with the mass spectroscopy determination.

	[μM]	pNATOG		[μM]	pNATOG
Neg	1.34	5.87	Pos	1.10	5.96
Neg	8.61	5.07	Pos	8.97	5.05
Neg	11.01	4.96	Pos	15.86	4.80
Neg	14.81	4.83	Pos	17.34	4.76
Neg	16.62	4.78	Pos	18.65	4.73
Neg	18.18	4.74	Pos	20.44	4.69
Neg	18.80	4.73	Pos	28.06	4.55
Neg	22.69	4.64	Pos	28.69	4.54
Neg	24.90	4.60	Pos	29.64	4.53
Neg	**38.96**	**4.41**	Pos	33.45	4.48
Neg	**39.58**	**4.40**	Pos	36.60	4.44
Neg	**42.23**	**4.37**	Pos	37.21	4.43
Neg	**51.17**	**4.29**	Pos	48.46	4.31
			Pos	49.95	4.30

Equation S1: Log-linear Gaussian distribution of *O. volvulus* mono-infection (N = 293, red) and *O. volvulus* negative control (N = 255, blue). Each volunteer urine sample had NATOG quantified by LC-MS. NATOG molar concentrations were converted into negative log concentrations and grouped in 0.5 p[NATOG] bins. The distribution was fit with a Gaussian curve utilizing a normalization parameter (N), the standard deviation (σ), the average (μ), and bin frequency (x).

$$Frequency = \frac{N}{\sqrt{2\pi\sigma^2}} * e^{\frac{-(x-\mu)^2}{2\sigma^2}}$$

Ethics Statement.

Ghana: Ethical clearance for the use of archived samples for biomarker research was granted by the Committee on Human Research Publication and Ethics at the University of Science and Technology in Kumasi, Ghana.

Cameroon: Ethical clearance for the use of archived samples for biomarker research was granted by the National Institutional Review board, Yaoundé and Administrative clearance from the Delegation of Public Health, South West Region. Approval for the study was granted by the National Ethics Committee of Research for Human Health in Cameroon.

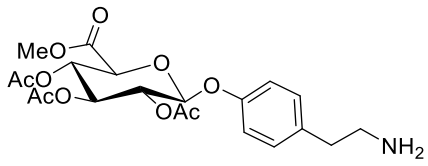
Germany: The use of archived samples was approved by the Ethics Committee at the University Hospital of Bonn, Germany.[1]

Sample collections and studies were performed in accordance with the Helsinki Declaration of 1975 as revised in 1983, 2000 and 2002. The Scripps Health Human Subjects Committee approved the use of all human urine samples in the study. These samples were determined by the Scripps Health Institutional Review Board (IRB) to be exempt from formal review under 45 Code of Federal Regulations 46 101. Onchocerciasis-positive samples were collected from endemic areas, and were confirmed by either skin snip (mf-positive) or nodule palpation (nodule-positive). Several samples included in this study were obtained during previously published research. Cameroon samples were collected as part of a nodulectomy campaign in villages surrounding Kumba, Cameroon, in 2006 and consist of urine from *O. volvulus*-positive individuals (nodule-positive with nodules containing live female worms), *O. volvulus*-negative individuals (skin snip-negative volunteers with no current or prior symptoms of *O. volvulus* infection).[2]

COMPOUND SYNTHESIS.

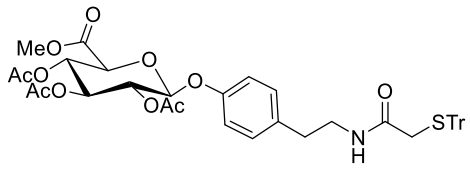
General Methods. Starting materials, reagents, and solvents were purchased from commercial vendors (Sigma Aldrich, Combi-Blocks, and Toronto Research Chemicals) unless otherwise noted. ^{1}H NMR spectrum were measured on 400, 500, and 600 MHz NMR instruments. The chemical shifts were reported at δ ppm relative to TMS using the residual solvent peak as the reference. The following abbreviations denote the peak multiplicities: s=single; d=doublet; dd=doublet of doublets; t=triplet; tt=triplet of triplets q=quartet; m=multiplet; br=broad. All reactions were monitored by HPLC-MS and thin layer chromatography (TLC) carried out on Merck (0.25 mm thick, $60F_{254}$) and visualized by UV (254 nm) or dyes such as *p*-anisaldehyde. Where necessary, a Teltdyne ISCO Combiflash Rf+ Lumen column chromatography system was used to purify mixtures with reagent-grade solvents.

DG1 Hapten Synthesis.



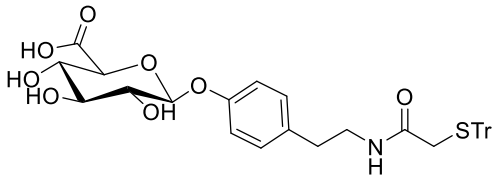
(Tyramine-O-(tri-O-acetyl-β-glucuronide methyl ester). *tert*-butyl (4-hydroxyphenethyl)carbamate (366.3 mg, 2.044 mmol) and the bromo glucuronic acid acceptor (1.084 g, 2.730 mmol) were dissolved in anhydrous dichloromethane (10 mL) and stirred for 30 min at room temperature over 4Å molecular sieves according to procedure reported in the literature.[1] Following pervious literature procedure, *N*-acetyltyramine-O-(tri-O-acetyl-β-glucuronide methyl ester) was synthesized from *N*-acetyltyramine (84.11 mg, 469 μmol) and the glucuronic acid donor (249.0 mg, 627 μmol) in the presence of silvertriflate (120.6 mg, 469 μmol) under anhydrous conditions.[3,4] Then silvertriflate (535.3 mg, 2.044 mmol) was added and the reaction mixture was stirred at room for 4 h. Afterward, the solution was diluted with dichloromethane and filtered through celite. The filtrate was extracted mixed with water and extracted with chloroform (4x), the organic layers were combined and dried over Na_2SO_4, filtered, and the

solvent was removed in vacuo. After dissolving the solid in anhydrous dichloromethane (10 mL), trifluoroacetic acid (469.5 μL, 6.131 mmol) was added and the solution was stirred for 1 h at room temperature. The solution was partitioned between dichloromethane and $NaHCO_3$ (sat.) and the organic fraction was separated, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by column chromatography elution using dichloromethane/methanol (97:3) yielded the desired product as a yellow powder (398.5 mg, 878.8 μmol, 43%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.12 (d, *J* = 8.0 Hz, 2H), 6.91 (d, *J* = 8.1 Hz, 2H), 5.37 (t, *J* = 9.3 Hz, 1H), 5.25 (m, *J* = 9.4, 5.5 Hz, 2H), 5.17 (d, *J* = 7.5 Hz, 1H), 4.24 (d, *J* = 9.7 Hz, 1H), 3.70 (s, 3H), 3.16 (d, *J* = 7.7 Hz, 2H), 2.90 (t, *J* = 7.6 Hz, 2H), 2.16 – 1.91 (m, 9H). HR-MS (ESI$^+$) calculated for $C_{21}H_{28}NO_{10}^+$ $(M + H)^+$: 454.17, found: 454.1720.



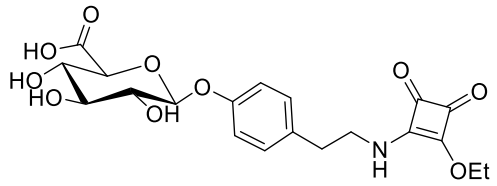
(2-(methoxycarbonyl)-6-(4-(2-(2-(tritylthio)acetamido)ethyl)phenoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate). Tyramine-O-(tri-O-acetyl-β-glucuronide methyl ester (385.0 mg, 849.1 μmol) and 2-(tritylthio)acetic acid (307.9 mg, 920.7 μmol) were added to a round bottle flask with anhydrous dichloromethane (60 mL). HOBt (240 mg, 1.567 mmol), EDC (266 mg, 1.388 mmol) and DIPEA (1mL) were added and the reaction stirred under argon at room temperature for 16 h. The reaction mixture was transferred to a separation funnel, partitioned and separated with $NaHCO_3$ (sat., 50ml) followed by NaCl (sat., 50mL). Dried organic layers were combined, dried over Na_2SO_4, filtered and solvent was removed in vacuo. The reaction mixture was purified by flash chromatography on a silica gel column. Elution with of 200:1 dichloromethane/methanol followed by 200:1:2 dichloromethane/methanol/triethylamine to give the product as a clear oil (251.9 mg, 327.2 μmol, 38.5%).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (d, *J* = 7.3 Hz, 6H), 7.29 – 7.26 (m, 6H), 7.25 – 7.20 (m, 3H), 7.03 (d, *J* = 8.3 Hz, 2H), 6.91 (d, *J* = 8.5 Hz, 2H), 6.00 (t, *J* = 5.2 Hz, 1H), 5.37 – 5.30 (m, 2H), 5.26 (dd, *J* = 15.5, 6.2 Hz, 1H), 5.06 (d, *J* = 7.4 Hz, 1H), 4.17 –

4.10 (m, 1H), 3.71 (s, 3H), 3.16 (tt, *J* = 12.8, 6.4 Hz, 2H), 2.58 (t, *J* = 7.2 Hz, 2H), 2.05 (t, *J* = 5.7 Hz, 9H). HR-MS (ESI$^+$) calculated for $C_{42}H_{44}NO_{11}S^+$ $(M + H)^+$: 770.26, found: 770.2630.



(3,4,5-trihydroxy-6-(4-(2-(2-(tritylthio)acetamido)ethyl)phenoxy)tetrahydro-2H-pyran-2-carboxylic acid). 2-(methoxycarbonyl)-6-(4-(2-(2-(tritylthio)acetamido)ethyl)phenoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (234.5 mg, 304 μmol) was dissolved in anhydrous methanol (6 mL) and was treated with NaOH (0.5 M, 3 mL) for 1.5 hrs. The solution was neutralized with HCl (1M) to precipitate pure hapten. The hapten was isolated by filtration to obtain a white powder (142.2 mg, 225.0 μmol, 74%). ^{1}H NMR (500 MHz, MeOD) δ 7.38 (d, *J* = 7.9 Hz, 6H), 7.29 (t, *J* = 7.5 Hz, 6H), 7.23 (t, *J* = 7.3 Hz, 3H), 7.09 (d, *J* = 8.7 Hz, 2H), 7.00 (d, *J* = 8.6 Hz, 2H), 4.90 (dd, *J* = 8.7, 5.2 Hz, 1H), 3.94 (d, *J* = 9.7 Hz, 1H), 3.64 – 3.58 (m, 1H), 3.53 – 3.43 (m, 2H), 3.19 (t, *J* = 7.2 Hz, 2H), 2.63 (t, *J* = 7.2 Hz, 2H). HR-MS (ESI$^+$) calculated for $C_{35}H_{35}NNaO_8S^+$ $(M + Na)^+$: 652.1978, found: 652.1978.

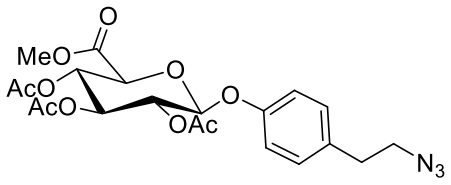
SQ1 Hapten Synthesis.



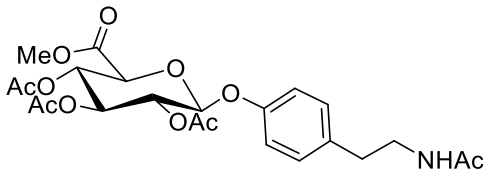
(Acetic 3,4,5-triacetoxy-6-(4-(2-((2-ethoxy-3,4-dioxocyclobut-1-en-1-yl)amino)ethyl)phenoxy)tetrahydro-2H-pyran-2-carboxylic anhydride). Tyramine-O-(tri-O-acetyl-*β*-glucuronide methyl ester (10 mg, 22.1 μmol) was dissolved in ethanol (1 mL) and cooled to 0^{o}C. NaOH (1 M, 120 μL) was added to the solution slowly and it was stirred at 0^{o}C for 1 h. The solution was then acidified with HCl (1M) and neutralized with $NaHCO_3$ (sat.). Triethylamine (15.4 μL, 110.3 μmol) was added to the solution followed

by diethyl squarate (18.76 mg, 110.3 μmol) and the mixture was stirred at 0°C for 2 h. The solution was then purified by reverse phase HPLC H_2O/ACN (0% to 95%) and the solvent was lyophilized to yield the product as a yellow oil (9.5 mg, 21.7 μmol, 98.5 %). ^{1}H NMR (500 MHz, Methanol-d4) δ 7.16 – 7.11 (m, 2H), 7.06 (dd, J = 8.0, 4.2 Hz, 2H), 4.73 (q, J = 7.1 Hz, 1H), 4.69 – 4.59 (m, 2H), 3.79 (t, J = 7.0 Hz, 1H), 3.75 (d, J = 8.9 Hz, 1H), 3.61 (t, J = 6.8 Hz, 1H), 3.55 – 3.44 (m, 3H), 2.84 (q, J = 6.7 Hz, 2H), 1.42 (dd, J = 7.1, 4.2 Hz, 3H). HR-MS (ESI$^+$) calculated for $C_{20}H_{23}NNaO_{10}^+$ $(M + Na)^+$: 460.12, found: 460.1215.

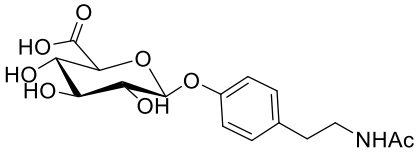
NATOG Synthesis.



(2-(4-(2-azidoethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate). The starting materials glycosyl trichloroacetimidate and 4-(2-azidoethyl)phenol were prepared and the reaction was run according to literature procedures.[5-7] The glycosyl trichloroacetimidate (1.1 g, 2.298 mmol) and 4-(2-azidoethyl)phenol (300 mg, 1.838 mmol) were dissolved in anhydrous dichloromethane, stirred over 4Å molecular sieves under argon, and cooled to -78°C. Boron trifluoride diethyl etherate (123.1μL, 0.9192 mmol) was added and the reaction stirred for 30 min at -78°C before allowing to warm to room temperature while stirring overnight. The reaction mixture was diluted with an equal volume of dichloromethane and washed with $NaHCO_3$ (sat.) thrice followed by brine. The organic phase was dried over Na_2SO_4, filtered, and the solvent was removed in vacuo. Purification by column chromatography elution using ethyl acetate/hexanes (1:1) yielded the desired product as a white powder (582.49 mg, 1.215 mmol, 66.1% yield). ^{1}H NMR (600 MHz, Chloroform-d) δ 7.14 (d, J = 8.6 Hz, 2H), 6.95 (d, J = 8.6 Hz, 2H), 5.37 – 5.31 (m, 2H), 5.29 – 5.24 (m, 1H), 5.11 (d, J = 7.4 Hz, 1H), 4.18 – 4.14 (m, 1H), 3.73 (s, 3H), 3.47 (t, J = 7.2 Hz, 2H), 2.84 (t, J = 7.2 Hz, 2H), 2.06 – 2.03 (m, 9H). HR-MS (ESI$^+$) calculated for $C_{21}H_{25}N_3NaO_{10}^+$ $(M + Na)^+$: 502.14, found: 502.1443.



(2-(4-(2-acetamidoethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate). 2-(4-(2-azidoethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (582.49 mg, 1.215 mmol) was dissolved in pyridine (4.89 mL), cooled to -30°C, and let stir under argon. Added thioacetic acid (9.67 mL, 133.6 mmol) slowly and the reaction stirred overnight. The solution was partitioned between ethyl acetate and $NaHCO_3$ (sat.) and was extracted with ethyl acetate (4 x 100 mL). The organic phases were combined, dried over Na_2SO_4, filtered, and the solution was concentration. The mixture was cooled to -10°C, filtered, and the precipitate was rinsed with diethyl ether to yield the desired product as a white powder (601 mg, 1.213 mmol, 99%). The analytical data are consistent with that reported previously.[3] ^{1}H NMR (600 MHz, Chloroform-d) δ 7.09 – 7.04 (m, 2H), 6.90 – 6.85 (m, 2H), 5.91 (s, 1H), 5.33 – 5.23 (m, 2H), 5.21 (m, J = 7.7, 6.2, 2.6 Hz, 1H), 5.10 (d, J = 8.0 Hz, 1H), 4.17 (d, J = 9.4 Hz, 1H), 3.67 (q, J = 3.1, 2.3 Hz, 3H), 3.38 (m, J = 11.0, 3.5 Hz, 2H), 2.70 (m, J = 7.1, 3.4 Hz, 2H), 2.02 – 1.97 (m, 9H), 1.87 (s, J = 3.2, 2.4 Hz, 3H).



(NATOG). 2-(4-(2-acetamidoethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (332.75 mg, 671.6 μmol) was dissolved in methanol, tetrahydrofuran, and H_2O (5:5:1, 15.62 mL), cooled to 0°C and stirred under nitrogen. K_2CO_3 (185.6 mg, 1.343 mmol) was added to the solution and it was stirred at 0°C overnight. The solution was neutralized with strongly acidic ion-exchange resin (Amberlyst. (R). 15 H+ Form). The mixture was filtered and the solvent was removed in vacuo to yield the desired product as a white powder (223.07 mg, 627.8 mmol, 93.5%). The analytical data are consistent with that reported previously.[5] ^{1}H NMR (600 MHz, Methanol-d4) δ 7.13 (d, J = 8.6 Hz, 2H),

7.06 (d, J = 8.6 Hz, 2H), 3.71 (d, J = 9.3 Hz, 1H), 3.56 – 3.46 (m, 3H), 3.35 (t, J = 7.3 Hz, 2H), 2.73 (t, J = 7.3 Hz, 2H), 1.90 (s, 3H).

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