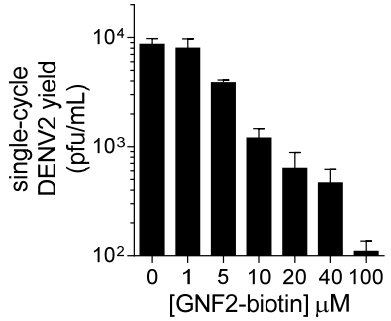
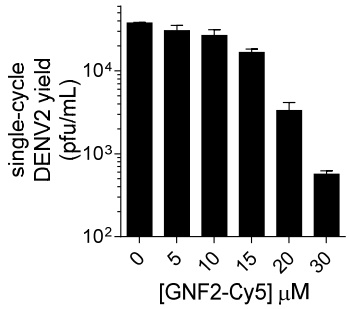
Figure S1. Related to Figures 3, 4, and 5. IC_{90} determinations for tool compounds GNF2-biotin, GNF2-Cy5, and GNF2-FITC. IC_{90} values were determined using the viral infectivity assay as described in Figure 2 in the main article. Briefly, DENV2 NGC virus was incubated with varying concentrations of compound for 45 minutes at 37 ºC, then added to cells and incubated for 60 minutes at 37 ºC to allow infection to occur. The inoculum was then removed and cells were washed to remove unbound virus and compound, and medium lacking inhibitor was added. Culture supernatants were collected at 24 hours post-infection, and the single-cycle yield of infectious particles was quantified by viral plaque formation assay as a metric of inhibition of viral infection 24 hours prior. The IC_{90} value was defined as the compound concentration sufficient to cause a 10-fold reduction in the single-cycle viral yield. Each compound titration was performed $n \geq 2$. Representative data shown are the mean ± standard deviation of triplicate plaque formation assays.

(A) GNF2-biotin



(B) GNF2-Cy5



(C) GNF2-FITC

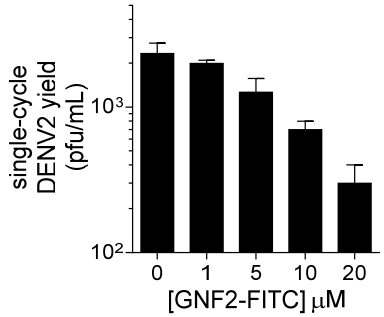
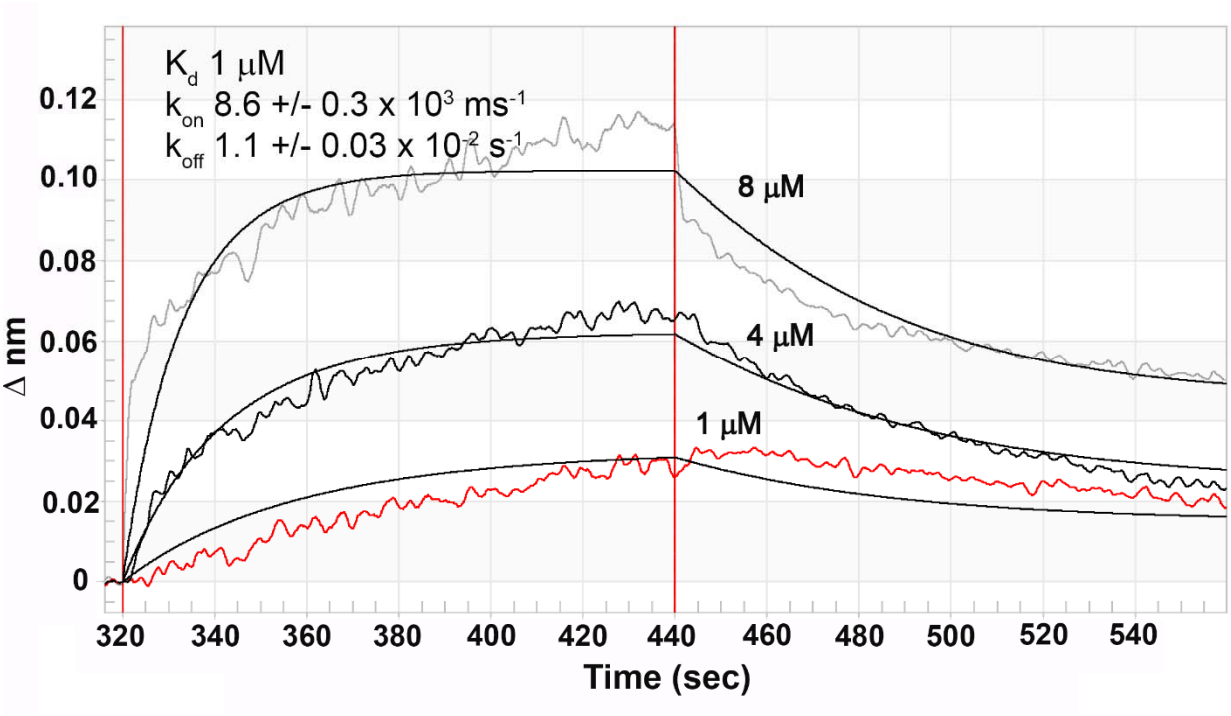


Figure S2. Related to Figure 5. Biolayer interferometry characterization of the interaction of GNF2-biotin with soluble, prefusion E. Streptavidin (SA) biosensor tips (ForteBio) were hydrated in running buffer (50 mM TAN, pH 8.0, with 150 mM NaCl and 0.1% Tween 20) for at least 10 minutes at room temperature and then blocked in blocking buffer (50 mM TAN, pH 8.0 with 150 mM NaCl and 5 ~ 10% FBS) for 3 to 10 minutes at room temperature prior to running Blitz experiments. SA tips were saturated with 25 µM GNF2-biotin or DMSO (as control) for 200-300 seconds. Baseline was then collected for 60 seconds in running buffer. Prefusion DENV2 sE at 1 to 8 µM was bound to the GNF2-biotin for 120 seconds and allowed to dissociate into running buffer for 120 seconds. The data were analyzed by both global and local (1:1) fitting using the ForteBioPro software.

Global fitting:

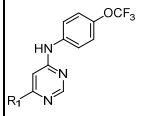
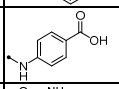
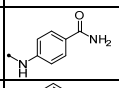
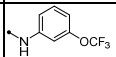
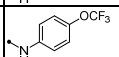
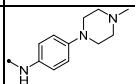
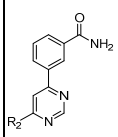
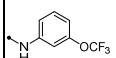
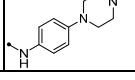
K_D (M)	k_a (ms^{-1})	k_a error	k_d (s^{-1})	k_d error
1.2e-6	8.6e2	2.8e2	1.1e-2	3.2e-4

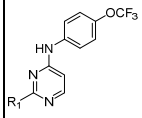
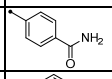
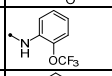
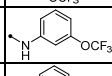
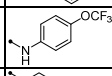
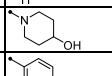
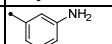
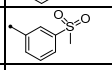
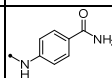
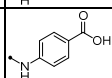
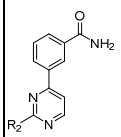
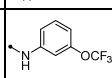


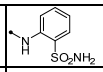
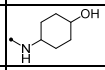
Local Fitting

sE concentration (µM)	K_D (M)	k_a (ms^{-1})	k_a error	k_d (s^{-1})	k_d error
1	2.307×10^{-6}	4.969×10^{3}	1.332×10^{2}	1.146×10^{-2}	1.654×10^{-4}
4	4.57×10^{-6}	7.955×10^{2}	1.148×10^{3}	3.635×10^{-3}	1.895×10^{-4}
8	2.43×10^{-6}	7.8×10^{3}	3.393×10^{2}	1.895×10^{-2}	6.475×10^{-4}

Table S1. Related to Table 1 and Figures 2 and 6B. Anti-DENV2 activity of (A) 4,6-disubstituted and (B) 2,4-disubstituted pyrimidines Columns are: 1. core chemical structure; 2. compound code; 3. chemical structure of R_1 or R_2 group; 4. Log unit decrease in plaque-forming units (pfu) relative to DMSO control at an inhibitor concentration of 25 µM relative to DMSO control. "ND" indicates that the compound exhibited no antiviral viral activity at 75 µM and no test at 25 µM was performed. "No inhibition" indicates that the compound at 25 µM caused no decrease in single-cycle viral yield when compared to the DMSO control. Compound **3m** exhibited no antiviral activity in the infectivity assay at concentrations up to 25 µM and was used as a negative control in the competitive Alphascreen assay (Figure 5B).

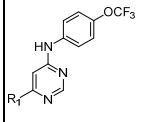
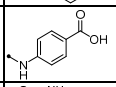
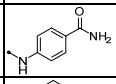
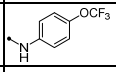
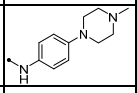
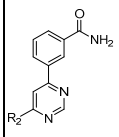
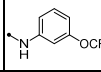
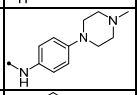
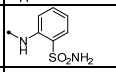
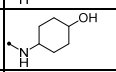
A	Compound	R1 or R2	pfu log_{10} unit decrease at 25 µM
	**3a** 1-100-1		1.1
	3b 1-097-3		0.8
	3c 1-100-2		0.3
	3d 1-093-3		0.2
	3e 2-001-1		ND
	3f 1-099		ND
	3g 1-093-2		ND
	3h 1-091		ND
	3i 1-096-1		ND
	3j 1-096-2		ND
	3k 1-093-1		ND
	3l 1-097-2		ND
	3m 2-10		ND
	**5a** 2-027-3		1.7
	5b 2-027-1		0.3
	5c 2-027-2		0.1
	5d 2-036-3		No inhibition
	5e 2-036-4		No inhibition

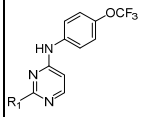
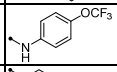
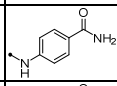
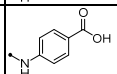
B	Compound	R1 or R2	pfu log_{10} unit decrease at 25 µM
	**8a** 2-033-2		4
	8b 2-012-2		4
	8c 2-012-3		4
	8d 2-21-2		1.1
	8e 2-023-3		1.0
	8f 2-012-4		0.9
	8g 2-023-2		0.8
	8h 2-037B		0.6
	8i 2-33-3		0.8
	8j 2-33-4		0.3
	8k 2-012-1		0.3
	8l 2-021-1		0.2
	8m 2-012-5		ND
	8n 2-012-6		ND
	8o 2-035		ND
	8p 2-023-1		ND
	8q 2-023-5		ND
	**10a** 2-043-3		0.52
	10b 2-043-2		0.27
	10c 2-42		ND
	10d 2-043-4		ND

	5f 2-036-5		No inhibition
	5g 2-036-1		No inhibition
	5h 2-036-2		No inhibition
	5i 2-027-5		No inhibition

	10e 2-043-1		ND

Table S2. Related to Table 1 and Figure 2. BCR-Abl activity of (A) 4,6-disubstituted and (B) 2,4-disubstituted pyrimidines Columns are: 1. core chemical structure; 2. compound code; 3. chemical structure of R_1 or R_2 group; 4. IC_{50} in μM for viability of BCR-Abl-dependent murine Ba/F3 cells.

A	Compound	R1 or R2	Bcr-Abl Ba/F3 IC_{50} (μM)
	**3a** 1-100-1		>10
	3b 1-097-3		1.8
	3c 1-100-2		>10
	3d 1-093-3		>10
	3e 2-001-1		>10
	3f 1-099		0.6
	3g 1-093-2		1.0
	3h 1-091		>10
	3i 1-096-1		>10
	3j 1-096-2		>10
	3k 1-093-1		>10
	3l 1-097-2		>10
	3m 2-10		1.0
	**5a** 2-027-3		6.4
	5b 2-027-1		>10
	5c 2-027-2		>10
	5d 2-036-3		>10
	5e 2-036-4		>10
	5f 2-036-5		>10
	5g 2-036-1		>10
	5h 2-036-2		>10
	5i 2-027-5		>10

B	Compound	R1 or R2	Bcr-Abl Ba/F3 IC_{50} (μM)
	**8a** 2-033-2		>10
	8b 2-012-2		>10
	8c 2-012-3		>10
	8d 2-21-2		>10
	8e 2-023-3		>10
	8f 2-012-4		>10
	8g 2-023-2		>10
	8h 2-037B		>10
	8i 2-33-3		>10
	8j 2-33-4		>10
	8k 2-012-1		>10
	8l 2-021-1		>10
	8m 2-012-5		>10
	8n 2-012-6		>10
	8o 2-035		>10
	8p 2-023-1		>10
	8q 2-023-5		>10
	10a 2-043-3		3.6
	10b 2-043-2		>10
	10c 2-42		1.9
	10d 2-043-4		3.2
	10e 2-043-1		>10

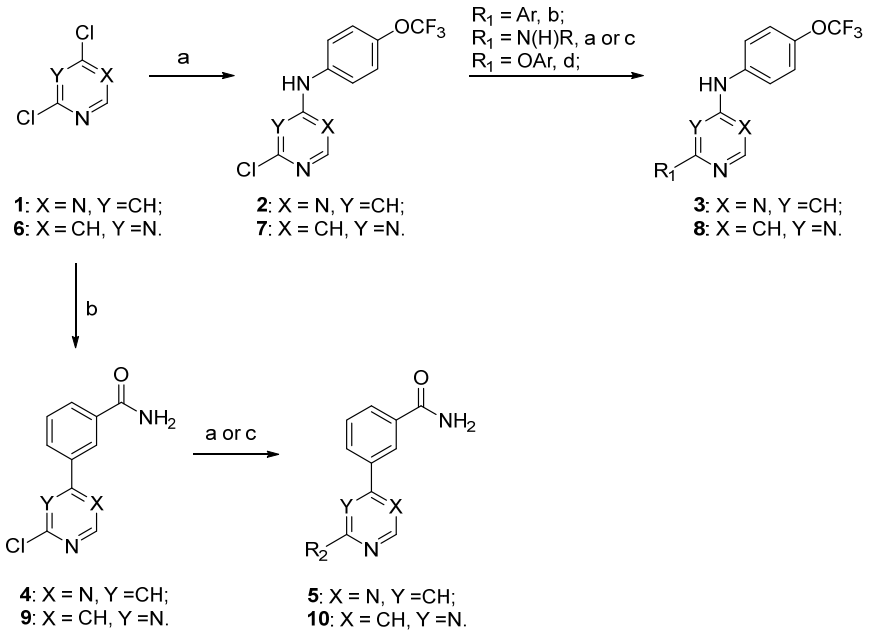
SUPPLEMENTAL EXPERIMENTAL PROCEDURES

1. Chemistry

Unless otherwise noted, reagents and solvents were obtained from commercial suppliers and were used without further purification. ^{1}H NMR spectra were recorded on 600 MHz (Varian AS600), and chemical shifts are reported in parts per million (ppm, δ) downfield from tetramethylsilane (TMS). Coupling constants (*J*) are reported in Hz. Spin multiplicities are described as s (singlet), brs (broad singlet), t (triplet), q (quartet), and m (multiplet). Mass spectra were obtained on a Waters Micromass ZQ instrument. Preparative HPLC was performed on a Waters Symmetry C18 column (19 x 50 mm, 5μM) using a gradient of 5-95% acetonitrile in water containing 0.05% trifluoacetic acid (TFA) over 8 min (10 min run time) at a flow rate of 30 mL/min. Purities of assayed compounds were in all cases greater than 95%, as determined by reverse-phase HPLC analysis.

2. General synthetic procedure of GNF-2 analogs.

Scheme S1.[a]



[a] Reagents and conditions: a) amine, DIEA, Dioxane, 90-110 °C, 12h; b) phenylboronic acid/ester, $Pd(PPh_3)_4$, Na_2CO_3(aq), Dioxane, 90-110 °C, 12h; c) amine, TFA or AcCl, isopropanol or ethylene glycol, 90-100 °C, 12h; d) phenol or 1-naphthalenol, NaH, Dioxane, 110 °C, 12h.

1). Amination with anilines or amines under basic conditions:

Mono or dichloro-pyrimidine (1 eq.), amine or aniline (1.5 eq.) and diisopropylethylamine (3 eq.) were dissolved in dioxane. The mixture was heated at 90 -110 °C for 12h. After removal of solvent by rotary evaportation, the product was purified using a suitable chromatographic separation method.

2). Coupling under Suzuki reaction conditions:

Mono or dichloro-pyrimidine (1 eq.), boronic acid or boronate ester (1.2 eq), tetrakis(triphenylphosphine)palladium (0.1 eq.) and 1N aqueous Na_2CO_3 (5 eq.) were dissolved in dioxane. The flask was degassed and purged with argon, and then stirred at 90 - 110 °C for 12h. The reaction mixture was diluted with ethyl acetate and filtered through a Celite pad followed by separation of organic and aqueous layers. The organic layer was washed with water and saturated brine solution, and then dried with anhydrous sodium sulfate. The product was purified by silica gel chromatography.

3). Amination with anilines under acidic conditions:

2-Chloro-4-substituted pyrimidine (1 eq.), aniline (3 eq.) and trifluoroacetic acid (3 eq.) were dissolved in isopropanol or ethylene glycol (for anilines insoluble in isopropanol). The mixture was heated at 90-100 °C for 12. Product was isolated in certain cases by addition of water followed by filtration of the precipitated solid. In the other cases, the product was purified using a suitable chromatographic separation method.

4). Alkylation with phenol and 1-naphthol:

Phenol or 1-Naphthol (1.5 eq) was treated with NaH (2 eq.) in dioxane at room temperature for 15 mins. Monochloro-pyrimidine (1 eq.) was added, and the reaction mixture was heated at 110 °C for 12. After addition of methanol, the solvents were removed on by rotary evaporation and the product was purified using a suitable chromatographic separation method.

3a
^{1}H NMR (600 MHz, $CDCl_3$) δ 9.97 (br s, 1H), 8.34 (s, 1H), 7.43 (m, 2H), 7.36 (m, 2H), 7.28 (m, 3H), 7.11 (m, 2H), 6.24 (s, 1H).

3m
^{1}H NMR (600 MHz, DMSO-d6) δ 9.23 (s, 1H), 8.84 (s, 1H), 8.20 (s, 1H), 7.67 (d, J = 9.0 Hz, 2H), 7.28 (d, J = 9.0 Hz, 2H), 7.24 (d, J = 9.0 Hz, 2H), 6.89 (d, J = 9.0 Hz, 2H), 5.99 (s, 1H), 3.06 (t, J = 4.8 Hz, 3H), 3.06 (t, J = 4.8 Hz, 3H), 2.20 (s, 3H).

8b
^{1}H NMR (600 MHz, $CDCl_3$) δ 8.41 (dd, J = 9.0, 1.2 Hz, 1H), 8.04 (d, J = 5.4 Hz, 1H), 7.35 (m, 2H), 7.15-7.20 (m, 4H), 6.94 (dt, J = 9.0, 1.8 Hz, 1H), 6.54 (br s, 1H), 6.14 (d, J = 6.0 Hz, 1H).

8c
^{1}H NMR (600 MHz, $CDCl_3$) δ 8.02 (d, J = 6.0, Hz, 1H), 7.67 (s, 1H), 7.36 (m, 2H), 7.26 (m, 1H), 7.21 (m, 1H), 7.16 (m, 3H), 6.80 (m, 1H), 6.55 (s, 1H), 6.11 (d, J = 6.0 Hz, 1H).

11a
^{1}H NMR (600 MHz, DMSO) δ 8.04 (d, J = 6.6 Hz, 1H), 7.77 (m, 1H), 7.69 (m, 1H), 7.46 (m, 1H), 7.42 (m, 3H), 7.37 (m, 3H), 7.01 (m, 1H), 6.39 (d, J = 6.6 Hz, 1H).

11b
^{1}H NMR (600 MHz, DMSO) δ 8.05 (d, J = 6.6 Hz, 1H), 7.76-7.73 (m, 3H), 7.42 (m, 3H), 7.34 (m, 2H), 7.30 (m, 2H), 6.53 (d, J = 6.6 Hz, 1H).

11c
^{1}H NMR (600 MHz, DMSO) δ 8.01 (m, 1H), 7.75 (m, 1H), 7.50 (d, J = 8.5 Hz, 1H), 7.40 (m, 1H), 7.32 (m, 2H), 7.23 (m, 4H), 6.40 (d, J = 6.0 Hz, 1H), 2.24 (s, 3H).

11d
^{1}H NMR (600 MHz, $CDCl_3$) δ 8.39 (d, J = 8.4 Hz, 1H), 8.09 (d, J = 6.0 Hz, 1H), 7.53 (m, 2H), 7.47 (m, 2H), 7.20 (m, 4H), 6.96 (m, 1H), 6.65 (s, 1H), 6.21 (d, J = 5.4 Hz, 1H).

11e
^{1}H NMR (600 MHz, DMSO) δ 8.00 (d, J = 6.6 Hz, 1H), 7.75 (m, 2H), 7.51 (m, 3H), 7.47 (m, 2H), 7.41 (m, 1H), 7.27 (m, 2H), 6.39 (d, J = 6.6 Hz, 1H).

11f
^{1}H NMR (600 MHz, DMSO) δ 7.98 (d, J = 6.6 Hz, 1H), 7.81 (m, 1H), 7.50 (m, 4H), 7.44 (m, 1H), 7.40 (m, 2H), 7.26 (m, 2H), 7.01 (m, 1H), 6.40 (d, J = 6.6 Hz, 1H).

11g

^{1}H NMR (600 MHz, DMSO) δ 8.17 (br s, 1H), 8.05 (d, J = 6.6 Hz, 1H), 7.85 (m, 4H), 7.55 (m, 4H),7.50-7.41 (m, 4H), 6.50 (d, J = 6.6 Hz, 1H).

11h
^{1}H NMR (600 MHz, DMSO-d_6) δ 7.84 (br s, 1H), 7.77 (br s, 1H), 7.47 (m, 2H), 7.37 (m, 2H), 7.31 (m, 2H), 7.26 (m, 2H), 7.19 (m, 2H), 6.28 (d, J = 6.0 Hz, 1H), 4.47 (d, J = 6.0 Hz, 2H).

11i
^{1}H NMR (600 MHz, DMSO-d_6) δ 7.84 (br s, 1H), 7.75 (br s, 1H), 7.41 (m, 2H), 7.33 (m, 2H), 7.28 (m, 3H), 6.52 (m, 2H), 6.22 (d, J = 6.0 Hz, 1H), 4.46 (d, J = 6.0 Hz, 2H).

11j
^{1}H NMR (600 MHz, $CDCl_3$) δ 8.18 (br s, 1H), 7.58 (br s, 1H), 7.34 (m, 1H), 7.30 (m, 2H), 7.15 (m, 1H), 7.11 (m, 2H), 7.00 (m, 1H), 6.91 (m, 1H), 6.48 (d, J = 6.0 Hz, 1H).

12a
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.75 (br s, 1H), 7.66 (d, J = 7.2 Hz, 1H), 7.53 (br s, 1H), 7.39 (m, 1H), 7.34 (m, 1H), 7.30 (m, 1H), 7.25 (t, J = 7.8 Hz, 2H), 7.19 (m, 1H), 7.11 (m, 1H), 6.94 (m, 1H), 6.16 (d, J = 7.2 Hz, 1H).

12b
^{1}H NMR (600 MHz, DMSO) δ 8.02 (d, J = 6.6 Hz, 1H), 7.57 (br s, 2H), 7.53 (d, J = 8.5 Hz, 1H), 7.39 (m, 1H), 7.31 (m, 2H), 7.28 (t, J = 7.8 Hz, 1H), 7.20 (m, 1H), 6.94 (d, J = 8.4 Hz, 1H), 6.38 (d, J = 6.0 Hz, 1H), 2.24 (s, 3H).

12c
^{1}H NMR (600 MHz, DMSO) δ 8.13 (d, J = 6.2 Hz, 1H), 7.98 (m, 2H), 7.87 (m, 3H), 7.65 (m, 2H), 7.56 (d, J = 7.8 Hz, 1H), 7.43 (t, J = 7.8 Hz, 1H), 7.00 (d, J = 7.8 Hz, 1H), 6.41 (d, J = 6.2 Hz, 1H).

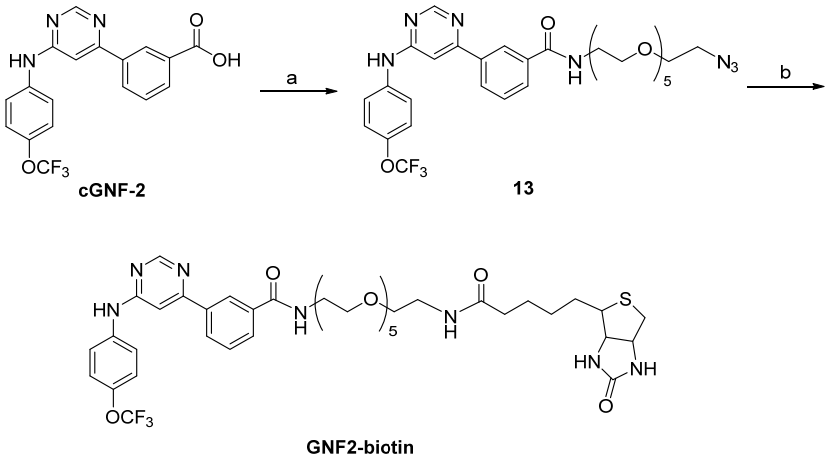
12d
^{1}H NMR (600 MHz, DMSO) δ 8.04 (d, J = 6.6 Hz, 1H), 7.98 (m, 2H), 7.80 (m, 1H), 7.65 (m, 2H), 7.55 (d, J = 7.8 Hz, 1H), 7.41 (m, 1H), 7.35 (m, 2H), 6.98 (br s, 1H), 6.34 (d, J = 6.2 Hz, 1H).

12e
^{1}H NMR (600 MHz, DMSO) δ 8.03 (d, J = 6.0 Hz, 1H), 7.79 (m, 2H), 7.60 (m, 3H), 7.42 (t, J = 8.4 Hz, 1H), 7.35 (m, 3H), 7.12 (m, 1H), 7.00 (br s, 1H), 6.37 (d, J = 6.0 Hz, 1H).

12f
^{1}H NMR (600 MHz, DMSO) δ 8.29 (br s, 1H), 8.06 (d, J = 6.6 Hz, 1H), 7.87 (d, J = 8.4 Hz, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.75 (m, 2H), 7.66 (m, 3H), 7.48 (m, 1H), 7.40 (m, 3H), 7.00 (m, 1H), 6.44 (d, J = 6.6 Hz, 1H).

3. Synthetic procedure of GNF2-biotin.

Scheme S2.[a]



[a] Reagents and conditions: (a) 17-azido-3,6,9,12,15-pentaoxaheptadecan-1-amine, HATU, DIEA, DMF; (b) (1) H_2, Pd/C, MeOH, (2) NHS-Biotin, DIEA, DMF.

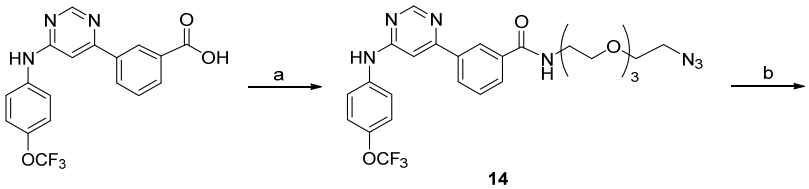
A reaction mixture of compound **cGNF-2** 3-(6-(4-(trifluoromethoxy)phenylamino)pyrimidin-4-yl)benzoic acid (61 mg, 0.18 mmol), 17-azido-3,6,9,12,15-pentaoxaheptadecan-1-amine (56 mg, 0.15 mmol), *O*-(7-Azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HATU) (86 mg, 0.23 mmol), and DIEA (78 □L, 0.45 mmol) in DMF (3 mL) was stirred at room temperature overnight. Once the reaction was completed, the reaction mixture was diluted with ethyl acetate (20 mL) and washed with brine (20 mL). The organic phase was dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography to afford the desired product **13** N-(17-azido-3,6,9,12,15-pentaoxaheptadecyl)-3-(6-(4-(trifluoromethoxy)phenylamino)pyrimidin-4-yl)benzamide (57 mg, 58% yield).

To a solution of compound **13** N-(17-azido-3,6,9,12,15-pentaoxaheptadecyl)-3-(6-(4-(trifluoromethoxy)phenylamino)pyrimidin-4-yl)benzamide (17 mg, 0.026 mmol) in 5 mL of methanol was added 5 mg Pd/C. The resulting suspension was degassed three times and then was stirred with a balloon filled with H_2 for 1 hour. The crude reaction mixture was filtered through a short pad of Celite, which was washed with 10 mL of methanol. The combined filtrates were concentrated and used for the next step without further purification.

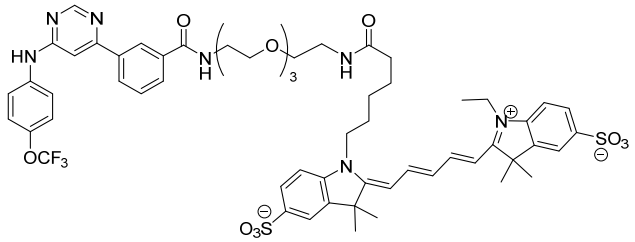
To a solution of above amine in DMF (2 mL) were added NHS-Biotin (Thermo Scientific), 2,5-dioxopyrrolidin-1-yl 5-(2-oxo-hexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoate, 8.8 mg, 0.026 mmol) and DIEA (4.5 □L, 0.026 mmol). After stirring at room temperature for 2 h, the reaction mixture was concentrated and purified by prep-HPLC to afford the title compound **8** (6.8 mg, 31% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.97 (s, 1H), 8.75 (s, 1H), 8.70 (t, J = 6.0 Hz, 1H), 8.51 (s, 1H), 8.17 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 7.8 Hz, 1H), 7.83 – 7.85 (m, 2H), 7.80 (t, J = 7.8 Hz, 1H), 7.62 (t, J = 7.8 Hz, 1H), 7.33 – 7.35 (m, 2H), 6.39 (s, 1H), 6.33 (s, 1H), 5.74 (s, 1H), 4.26 – 4.30 (m, 1H), 4.09 – 4.13 (m, 1H), 3.49 – 3.57 (m, 20H), 3.36 (t, J = 6.0 Hz, 2H), 3.14 – 3.17 (m, 2H), 3.05 – 3.08 (m, 1H), 2.79 (dd, J = 12.0, 5.4 Hz, 1H), 2.53 – 2.57 (m, 1H), 2.04 (t, J = 7.2 Hz, 2H), 1.56 -1.60 (m, 1H), 1.41 – 1.49 (m, 3H), 1.24 – 1.30 (m, 2H).

4. Synthetic procedure of GNF2-CY5.

Scheme S3.[a]



cGNF-2 **14**



GNF2-Cy5

[a] Reagents and conditions: (a) 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethanamine, HATU, DIEA, DMF; (b) (1) H_2, Pd/C, MeOH, (2) Cy5-NHS ester, DIEA, DMF.

The reaction procedure is similar to the synthesis of GNF2-biotin. Cy5-NHS ester was purchased from GE Healthcare. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.98 (s, 1H), 8.75 (s, 1H), 8.67 (t, J = 8.4 Hz, 1H), 8.53 (s, 1H), 8.33 (m, 2H), 8.16 (m, 1H), 7.97 (m, 1H), 7.86 – 7.84 (m, 2H), 7.81 (m, 2H), 7.77 (t, J = 9.0 Hz, 1H), 7.63 (dd, J = 12.5 Hz, 1.8 Hz, 2H), 7.60 (t, J = 12.5 Hz, 1H), 7.41 (d, J = 1.2 Hz, 1H), 7.34 (d, J = 12.5 Hz, 2H), 7.30 (d, J = 12.5 Hz, 1H), 7.28 (d, J = 12.5 Hz, 1H), 6.55 (t, J = 18.6 Hz, 1H), 6.29 (d, J = 9.0 Hz, 1H), 6.25 (d, J = 9.0 Hz, 1H), 4.11-4.03 (m, 4H), 3.50-3.43 (m, 14H), 3.12 (q, J = 8.4Hz, 2H), 2.02 (t, J = 10.8 Hz, 2H), 1.65 (s, 12H), 1.50 (m, 3H), 1.25-1.21 (m, 6H).

5. Dengue virus purification

Virus was precipitated from C6/36 infected cell supernatant by addition of polyethylene glycol molecular weight 8000 (PEG8000) and incubation overnight at 4°C with constant mixing. The virus was then pelleted by centrifugation for 30 mins at 10,000×*g*, 4°C, and the pellet was resuspended in TAN buffer (20mM triethanolamine, 100mM NaCl, pH 8.0) and clarified by centrifugation at 5,000×*g* for 20 mins at 4°C. Virions were further purified on a 10% and 40% potassium tartrate with 7.5 and 30% glycerol, step gradient and centrifuged at 146,000×*g* for 2.5 hrs at 4°C. The gradient was harvested into 10 fractions and immunoblotted with monoclonal antibody 4G2 against E (D1-4G2-4-15, ATCC® HB-112™) or monoclonal antibody 6F3.1 against core (Bulich and Aaskov, 1992) to locate the virus. Those fractions were then pooled, concentrated and cleared of potassium tartrate and glycerol by exchange into TAN buffer using a 100K molecular weight cut-off concentrator (Amicon). Virus concentration was determined with the BCA assay (Pierce). Purified virus was stored at 4°C and used within one week of purification.

References for Supporting Materials and Methods

Bulich, R., and Aaskov, J.G. (1992). Nuclear localization of dengue 2 virus core protein detected with monoclonal antibodies. J Gen Virol 73 (Pt 11), 2999-3003.