Supporting Information

Facile transformation of rofecoxib into a novel near-infrared AIEgen for real-time dynamic monitoring of lipid droplets metabolism

Instruments.

High-resolution mass spectra (HRMS) were obtained at the Mass Spectrometry Service Center of Fujian institute of microbiology on an Agilent 6545 Q-TOF LCMS. Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker UNI-500 and AVII-500B instrument and JEOL 600MHz spectrometers (Framingham, MA, USA) using deuterated solvent (DMSO-*d6*). Photoluminescence (PL) excitation and emission spectra and PL decays were recorded on the FLS980 spectrometer (Edinburgh) equipped with both continuous Xenon (450 W). PL, AIE were taken by using a Canon 70D digital camera without using any filter. All the spectral data were recorded at room temperature unless otherwise noted, and corrected for the spectral response of both the spectrometer and the integrating sphere.

Materials and methods

General Information.

Unless otherwise stated, all chemicals and reagents were obtained commercially (Aladdin-Reagent and Damas-Beta) and used without further purification. All of the solvents were purified and dried before use by conventional methods.

Cell lines

Cell lines (human cervical adenocarcinoma cell line **Hela**, mouse embryonic fibroblast cell lines **3T3-L1**) were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences. 3T3-L1 cells were maintained in NGM medium (NGM: DMEM with 1% Glutamax, 1% Non-essential Amino Acids and 1 mM sodium pyruvate) supplemented with 5% newborn calf serum. Hela cells were maintained in 1640 medium supplemented with 10% fetal bovine serum (Gibco, Invitrogen, Grand Island, NY, USA), 100 μg/mL penicillin and 100 μg/mL streptomycin (Gibco, Invitrogen, Grand Island, NY, USA) at 37℃with 5% CO_2.

Cytotoxicity Assays

Hela cells were seeded into 96-well microplates at 1×10^4 cells per well. 18 h later, these adhesive cells were incubated with RFHX-1 or BODIPY 493/593 at various concentrations (0, 1, 10, 100 μM). The background control wells in the plate were filled only with culture medium. After an incubation of 24 h, cells were washed with sterile PBS before fresh medium was added. Subsequently, the cells were treated with 5 mg/mL MTT (10 μL/well) and incubated for an additional 4 h (37 °C, 5% CO_2). The cells were then dissolved in dimethyl sulfoxide (150 μL/well), and the absorbance at 570 nm was recorded using a

microplate reader (Biotek, USA).

Hela cells treatment with oleic acid

HeLa cells were grown on a 35mm Petri dish overnight; the cells were incubated with oleic acid (OA) (0.1 mM) and without OA as control at different times (0-24 h) and subsequently stained with RFHX-1 or BODIPY 493/593 (10 μM) for 20 min.

Differentiation of 3T3-L1 adipocytes

Differentiation of 3T3-L1 adipocytes was performed by changing the growth medium to NGM medium with 1 μM insulin, supplemented with 100 nM dexamethasone and 500 μM 3-isobutyl-1-methylxanthine for 2 days, followed with another 2-day culturing in NGM medium with 1 μM insulin. After that, cells were returned back to NGM medium for at least 12 days before starting the detection experiments. And subsequently stained with RFHX-1 or BODIPY 493/593 (10 μM) for 20 min.

Cell imaging and Co-localization

Cell imaging was taken using a confocal laser-scanning microscope (Olympus FluoViewTM FV1000, Japan). Hela cells in 1640 medium (5×10^4/mL, 1 mL) were plated onto a confocal chamber and incubated at 37 ℃ for 18 h to allow cell adhesion. To the culture medium was added RFHX-1 and BODIPY 493/593 (10 μM). After 20 min incubation, the chamber was gently washed with PBS buffer to remove unbound. An incubation chamber was applied for live-cell and Real-Time (RT) imaging, which was connected to temperature control unit 37 °C and CO_2 controller (1-2 hours before the experiment was allowed for stabilization of the temperature and CO_2 concentration).

Flow cytometry

Six medium plates of HeLa cells were grown overnight. Then the cells were incubated with 100 μM of oleic acid for 0, 2, 3 and 4 h before collection for flow cytometry. The cells were stained with 10 μM of RFHX-1 or BODIPY 493/593 for 15 min, respectively, and washed with PBS. The cells were analyzed by flow cytometry (Becton Dickinson FACS Aria IIIu). 10000 events were taken for each trial.

Photostability

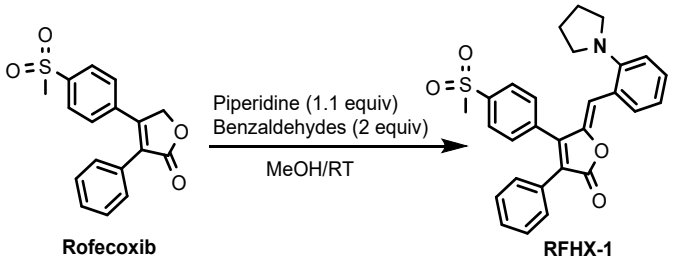
On a confocal microscope (Leica DMI 6000 Fully Motorized Inverted Microscope), the dyes were excited with 488 nm laser light for one-photon imaging. Imaging parameters were set for each dye individually to obtain optimal images. Repeated image scans were taken. On each series of scans, six regions of interest (ROIs) with several LDs were defined. The first scan of each ROI was set to 100%. Then the pixel intensity values for each ROI were averaged and plotted against the scan number. The

resulting curve represented the bleaching rate that an experimentalist would encounter.

Statistical analysis.

All data represent group means and standard errors of the mean (SEM). Individual group means were compared by the Newman-Keuls multiple-range test. GB-STAT software (Dynamic Microsystems, Inc., Silver Spring, MD, USA) was used for all statistical analyses

Synthesis and characterization of RFHX-1: (5Z)-4-(4-(methylsulfonyl)phenyl)-3-phenyl-5-(2-(pyrrolidin-1-yl)benzylidene)furan-2(5H)-one



Scheme-S1. The synthetic route of RFHX-1

Preparation: Rofecoxib (0.4 g, 1.3 mmol) and 2-cyanobenzaldehyde (2.6 mmol) were dissolved in 15 mL of methanol. To this solution, piperidine (0.12 g, 1.4 mmol) was added, and the mixture was stirred at room temperature under dark conditions for 12 hours. After completion, the reaction mixture was cooled, and the precipitate was filtered off and washed with methanol. An orange solid powder, RFHX-1, was obtained in 71% yield.

^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.05 (d, J = 8.7 Hz, 2H), 7.85 (d, J = 9.9 Hz, 1H), 7.69 (d, J = 8.7 Hz, 2H), 7.37 - 7.28 (m, 5H), 7.25 - 7.14 (m, 1H), 6.99 - 6.86 (m, 2H), 6.02 (s, 1H), 3.28 (s, 3H), 3.11 - 2.98 (m, 4H), 1.75 - 1.71 (m, 4H). ^{13}C-NMR (101 MHz, DMSO-*d6*) δ 168.3, 150.3, 149.5, 146.5, 142.2, 136.0, 131.6, 130.7, 130.6, 129.4, 129.4, 129.0, 128.0, 124.9, 123.0, 120.6, 116.8, 112.3, 52.6, 43.7, 24.9.

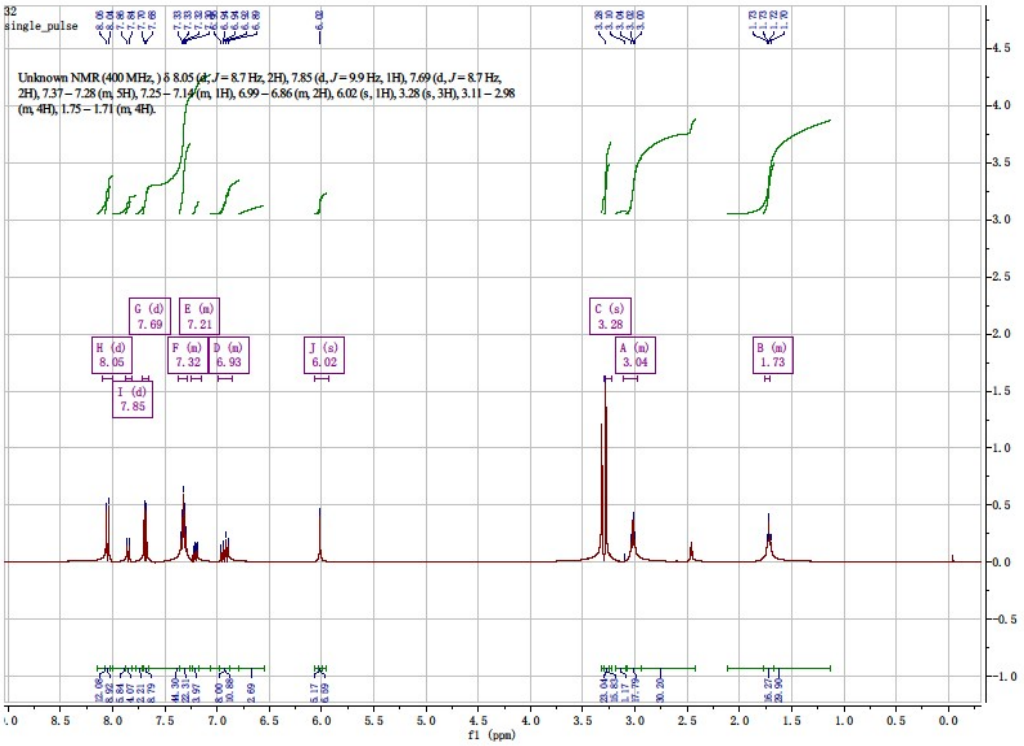


Figure S1. ^{1}H NMR (400 MHz, DMSO-d_6) for RFHX-1

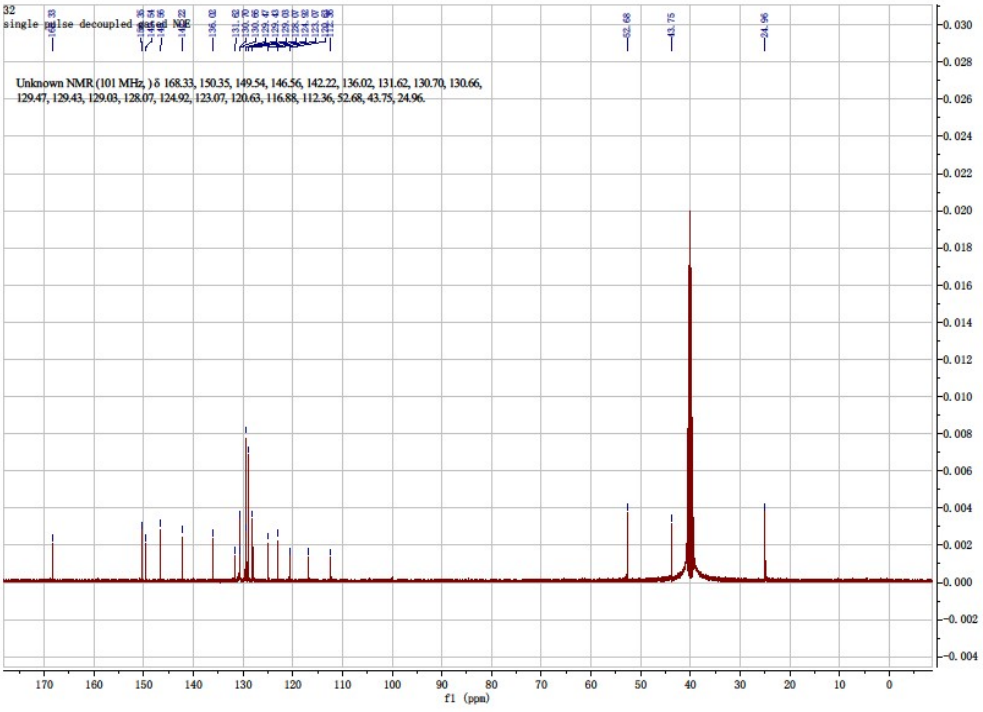


Figure S2. ^{13}C NMR (101 MHz, DMSO-d_6) for RFHX-1

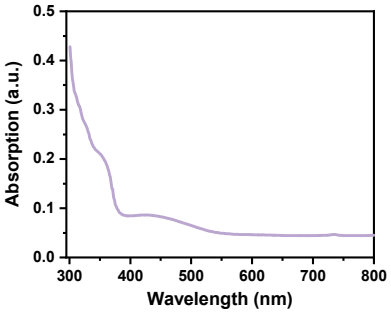


Figure S3. The Absorption spectrum of RFHX-1 (10 μM)

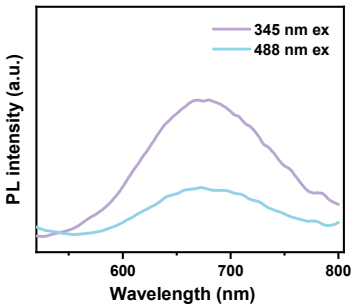


Figure S4. The Emission spectra of RFHX-1 (10 μM in DMSO, λ_{ex} = 345 nm and 488 nm)

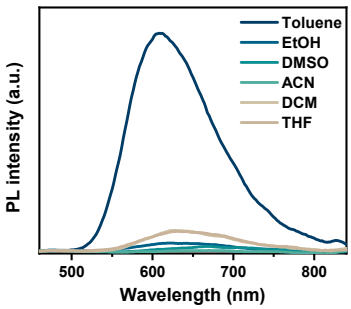


Figure S5. The Emission spectra of RFHX-1 (50 μM in different solvents, λ_{ex} = 365 nm)

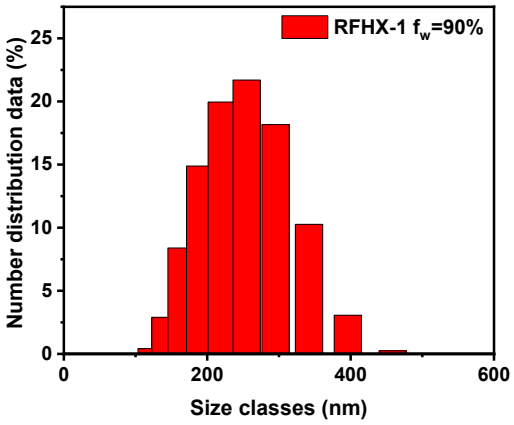


Figure S6. Particle size distribution of RFHX-1 in DMSO/H2O (fw = 90%)

The two-photon absorption cross section (б2PA) of RFHX-1

An open-aperture Z-scan experiment was used to investigate the sign and magnitude of the nonlinear absorption coefficient of the compounds. In femtosecond Z-scan measurements, the input wavelength outputs from the OPA were tuned to be 800 nm. The repetition rate was tuned to be 20 Hz. All Z-scan experiments were performed under a nearly Gaussian beam. A basic description of the experimental apparatus can be found in ref[32]. All of the compounds were dissolved in toluene at a concentration of 10×10^{-3} mol L^{-1} and placed in 2 mm quartz cells.

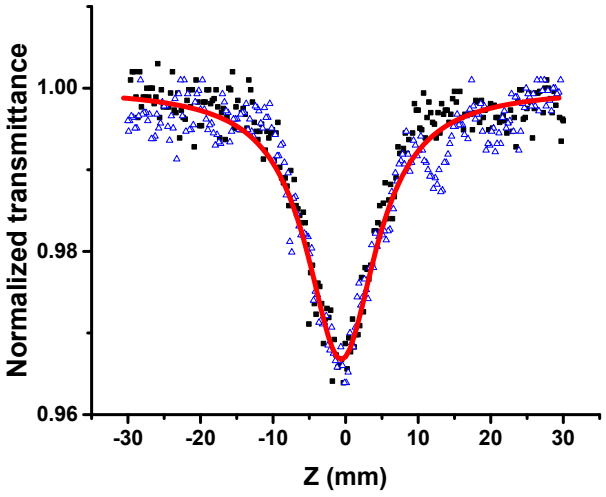


Figure S7. Femtosecond open-aperture Z-scan experiments at 800 nm. Dots show experimental data and the solid lines show fitted curves.

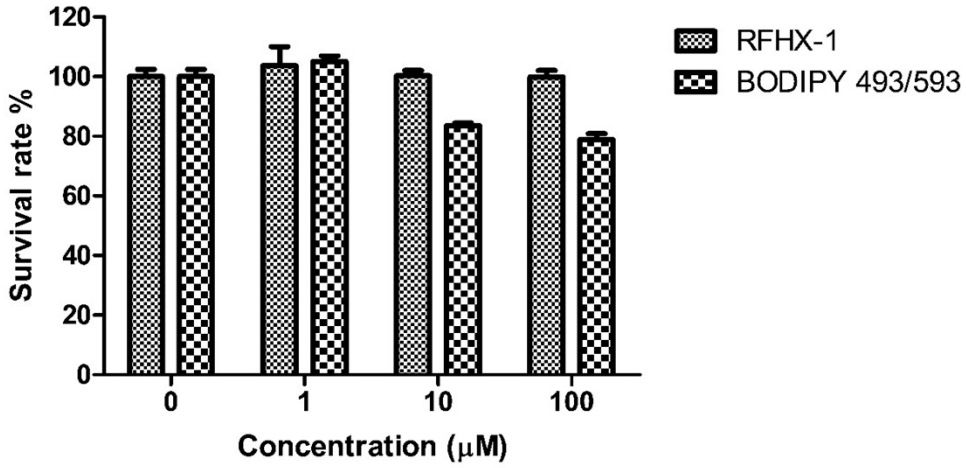


Figure S8. Using MTT assay to detect toxicity of RFHX-1 and BODIPY 493/593 for Hela cells under different concentrations.

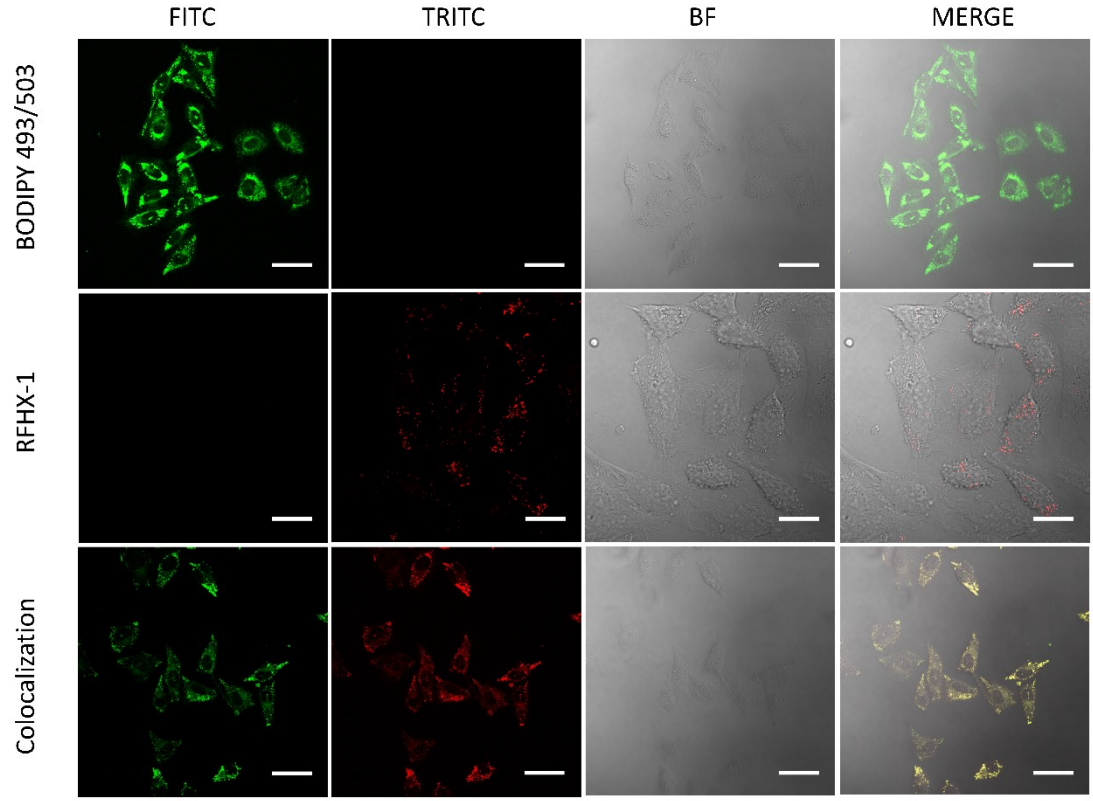


Figure S9. Fluorescence images of HeLa cells stained with RFHX-1 and BODIPY 493/593 (10 μM, λ_{ex} = 488 nm, λ_{em} = 570-1000 nm), scale bar: 20 μm, corresponding to a colocalization coefficient of 0.93.

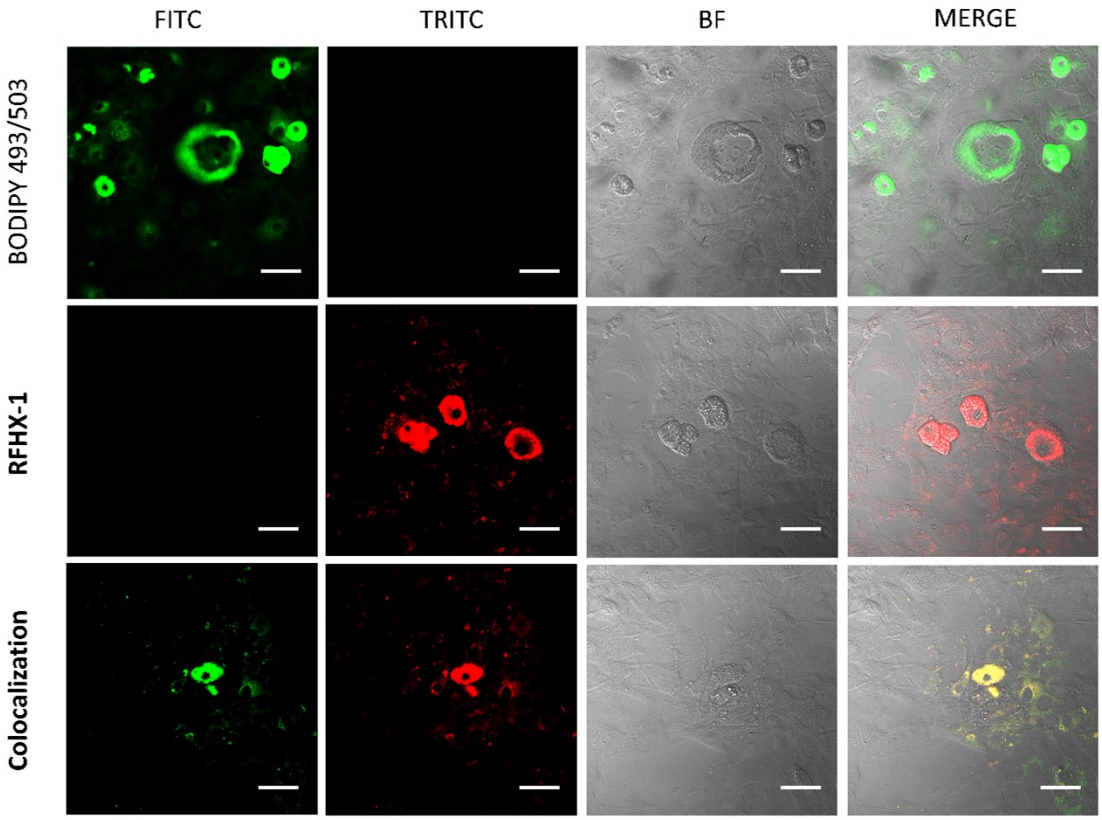


Figure S10. Fluorescence images of 3T3-L1 cells stained with RFHX-1and BODIPY 493/593 (10 μM, λ_{ex} = 488 nm, λ_{em} = 570-1000 nm), scale bar: 20 μm.

Crystal data and the collection of parameters

1. Crystal data and structure refinement for RFHX-1.

Identification code	RFHX-1
Empirical formula	C28 H25 N O4 S
Formula weight	471.55
Temperature	150(2) K
Wavelength	1.54178 A
Crystal system, space group	Monoclinic, P2/c
Unit cell dimensions	a = 17.8670(4) A alpha = 90 deg. b = 6.04330(10) A beta = 96.539(2) deg.

	c = 21.7935(4) A gamma = 90 deg.
Volume	2337.86(8) A^3
Z, Calculated density	4, 1.340 Mg/m^3
Absorption coefficient	1.521 mm^{-1}
F(000)	992
Crystal size	0.100 x 0.060 x 0.050 mm
Theta range for data collection	4.083 to 72.290 deg.
Limiting indices	-22<=h<=22, -7<=k<=5, -26<=l<=25
Reflections collected / unique	10416 / 4503 [R(int) = 0.0306]
Completeness to theta = 67.679	99.7 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	1.00000 and 0.59483
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4503 / 0 / 307
Goodness-of-fit on F^2	1.020
Final R indices [I>2sigma(I)]	R1 = 0.0489, wR2 = 0.1267
R indices (all data)	R1 = 0.0611, wR2 = 0.1398
Extinction coefficient	n/a
Largest diff. peak and hole	0.831 and -0.462 $e.A^{-3}$

2. Atomic coordinates (x 10^4) and equivalent isotropic
displacement parameters (A^2 x 10^3) for RFHX-1.
U(eq) is defined as one third of the trace of the orthogonalized
Uij tensor.

	x	y	z	U(eq)
S(1)	4249(1)	4893(1)	3562(1)	31(1)
O(1)	1493(1)	3242(2)	367(1)	24(1)
O(2)	1618(1)	5601(3)	-414(1)	32(1)
O(3)	4982(1)	3951(4)	3559(1)	49(1)
O(4)	4180(1)	7108(3)	3790(1)	46(1)
N(1)	1350(1)	-1133(3)	2391(1)	24(1)
C(1)	1833(1)	5016(3)	104(1)	24(1)
C(2)	2457(1)	5811(3)	551(1)	21(1)
C(3)	2469(1)	4481(3)	1056(1)	21(1)
C(4)	1868(1)	2883(3)	951(1)	22(1)
C(5)	1673(1)	1336(3)	1347(1)	22(1)
C(6)	1105(1)	-375(3)	1282(1)	22(1)
C(7)	700(1)	-905(4)	708(1)	28(1)
C(8)	239(1)	-2741(4)	636(1)	30(1)
C(9)	184(1)	-4127(4)	1133(1)	31(1)
C(10)	558(1)	-3628(3)	1709(1)	27(1)
C(11)	998(1)	-1706(3)	1803(1)	23(1)
C(12)	1197(2)	1058(4)	2652(1)	43(1)
C(13)	1185(2)	732(5)	3330(1)	52(1)
C(14)	1642(2)	-1384(4)	3467(1)	42(1)
C(15)	1395(1)	-2759(4)	2896(1)	31(1)
C(16)	2956(1)	4606(3)	1651(1)	20(1)
C(17)	3413(1)	2832(3)	1866(1)	24(1)
C(18)	3827(1)	2929(4)	2444(1)	26(1)
C(19)	3776(1)	4805(3)	2805(1)	23(1)
C(20)	3336(1)	6596(3)	2591(1)	27(1)
C(21)	2934(1)	6499(3)	2010(1)	26(1)
C(22)	3713(2)	3119(5)	3982(1)	51(1)
C(23)	2959(1)	7635(3)	424(1)	22(1)
C(24)	3710(1)	7665(4)	687(1)	25(1)
C(25)	4180(1)	9407(4)	573(1)	28(1)
C(26)	3910(1)	11139(4)	190(1)	30(1)
C(27)	3170(1)	11102(4)	-82(1)	28(1)
C(28)	2694(1)	9377(3)	34(1)	25(1)

3. Anisotropic displacement parameters (A^2 x 10^3) for RFHX-1.

	U11	U22	U33	U23	U13	U12
S(1)	30(1)	45(1)	17(1)	-2(1)	-3(1)	-4(1)
O(1)	27(1)	25(1)	19(1)	2(1)	-4(1)	-3(1)
O(2)	40(1)	35(1)	20(1)	5(1)	-7(1)	-4(1)
O(3)	31(1)	80(1)	32(1)	-6(1)	-10(1)	3(1)
O(4)	57(1)	50(1)	28(1)	-14(1)	-5(1)	-6(1)
N(1)	31(1)	19(1)	23(1)	1(1)	1(1)	-1(1)
C(1)	26(1)	23(1)	22(1)	2(1)	1(1)	0(1)
C(2)	22(1)	20(1)	19(1)	-1(1)	2(1)	2(1)
C(3)	21(1)	22(1)	20(1)	0(1)	2(1)	1(1)
C(4)	23(1)	22(1)	18(1)	0(1)	-2(1)	0(1)
C(5)	22(1)	24(1)	20(1)	-1(1)	-1(1)	0(1)
C(6)	22(1)	21(1)	24(1)	0(1)	2(1)	1(1)
C(7)	29(1)	26(1)	28(1)	1(1)	-1(1)	-1(1)
C(8)	29(1)	29(1)	31(1)	-6(1)	-4(1)	-2(1)
C(9)	28(1)	23(1)	40(1)	-5(1)	0(1)	-4(1)
C(10)	28(1)	22(1)	31(1)	1(1)	3(1)	-2(1)
C(11)	22(1)	21(1)	25(1)	1(1)	1(1)	0(1)
C(12)	76(2)	27(1)	27(1)	-3(1)	3(1)	6(1)
C(13)	75(2)	48(2)	32(1)	-6(1)	4(1)	1(2)
C(14)	55(2)	44(1)	25(1)	5(1)	-2(1)	-4(1)
C(15)	34(1)	28(1)	30(1)	7(1)	1(1)	-2(1)
C(16)	21(1)	22(1)	17(1)	1(1)	1(1)	-1(1)
C(17)	28(1)	22(1)	21(1)	-3(1)	0(1)	1(1)
C(18)	27(1)	26(1)	22(1)	4(1)	-2(1)	2(1)
C(19)	22(1)	30(1)	17(1)	0(1)	0(1)	-4(1)
C(20)	31(1)	24(1)	25(1)	-6(1)	2(1)	1(1)
C(21)	29(1)	24(1)	25(1)	0(1)	1(1)	3(1)
C(22)	60(2)	69(2)	23(1)	10(1)	2(1)	-15(2)
C(23)	26(1)	22(1)	16(1)	-1(1)	3(1)	0(1)
C(24)	29(1)	27(1)	18(1)	3(1)	2(1)	1(1)
C(25)	27(1)	33(1)	25(1)	1(1)	2(1)	-4(1)
C(26)	35(1)	27(1)	28(1)	-1(1)	6(1)	-6(1)
C(27)	36(1)	23(1)	24(1)	4(1)	5(1)	2(1)
C(28)	27(1)	25(1)	22(1)	1(1)	2(1)	2(1)

Table 4. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (A^2 x 10^3) for RFHX-1.

	x	y	z	U(eq)
H(5A)	1961	1375	1741	27
H(7A)	745	23	363	33
H(8A)	-38	-3051	247	36
H(9A)	-112	-5433	1080	37
H(10A)	515	-4602	2046	33
H(12A)	706	1636	2461	52
H(12B)	1596	2124	2573	52
H(13A)	663	548	3433	62
H(13B)	1421	1999	3567	62
H(14A)	2190	-1084	3508	50
H(14B)	1512	-2117	3847	50
H(15A)	1768	-3928	2837	37
H(15B)	899	-3457	2925	37
H(17A)	3440	1553	1617	29
H(18A)	4142	1731	2590	31
H(20A)	3312	7877	2840	32
H(21A)	2641	7733	1856	31
H(22A)	3207	3738	3990	76
H(22B)	3675	1660	3785	76
H(22C)	3958	2974	4406	76
H(24A)	3900	6480	945	30
H(25A)	4688	9418	757	34
H(26A)	4231	12340	115	36
H(27A)	2988	12270	-350	33
H(28A)	2186	9376	-152	30

Table 5. Torsion angles [deg] for RFHX-1.

C(4)-O(1)-C(1)-O(2)	177.80(19)
C(4)-O(1)-C(1)-C(2)	0.0(2)
O(2)-C(1)-C(2)-C(3)	-176.9(2)
O(1)-C(1)-C(2)-C(3)	0.6(2)
O(2)-C(1)-C(2)-C(23)	0.7(4)
O(1)-C(1)-C(2)-C(23)	178.19(17)
C(23)-C(2)-C(3)-C(4)	-178.27(19)
C(1)-C(2)-C(3)-C(4)	-0.9(2)
C(23)-C(2)-C(3)-C(16)	5.8(3)
C(1)-C(2)-C(3)-C(16)	-176.77(19)
C(1)-O(1)-C(4)-C(5)	177.70(19)
C(1)-O(1)-C(4)-C(3)	-0.5(2)
C(2)-C(3)-C(4)-C(5)	-177.2(2)
C(16)-C(3)-C(4)-C(5)	-1.0(3)
C(2)-C(3)-C(4)-O(1)	0.9(2)
C(16)-C(3)-C(4)-O(1)	177.14(16)
O(1)-C(4)-C(5)-C(6)	4.7(4)
C(3)-C(4)-C(5)-C(6)	-177.5(2)
C(4)-C(5)-C(6)-C(7)	9.7(3)
C(4)-C(5)-C(6)-C(11)	-176.2(2)
C(11)-C(6)-C(7)-C(8)	-3.6(3)
C(5)-C(6)-C(7)-C(8)	170.5(2)
C(6)-C(7)-C(8)-C(9)	-1.5(3)
C(7)-C(8)-C(9)-C(10)	3.2(3)
C(8)-C(9)-C(10)-C(11)	0.2(3)
C(9)-C(10)-C(11)-N(1)	176.95(19)
C(9)-C(10)-C(11)-C(6)	-5.3(3)
C(15)-N(1)-C(11)-C(10)	12.7(3)
C(12)-N(1)-C(11)-C(10)	-124.1(2)
C(15)-N(1)-C(11)-C(6)	-165.04(19)
C(12)-N(1)-C(11)-C(6)	58.1(3)
C(7)-C(6)-C(11)-C(10)	6.8(3)
C(5)-C(6)-C(11)-C(10)	-167.52(18)
C(7)-C(6)-C(11)-N(1)	-175.38(18)
C(5)-C(6)-C(11)-N(1)	10.3(3)
C(11)-N(1)-C(12)-C(13)	143.8(2)
C(15)-N(1)-C(12)-C(13)	2.4(3)
N(1)-C(12)-C(13)-C(14)	22.6(3)
C(12)-C(13)-C(14)-C(15)	-38.1(3)
C(11)-N(1)-C(15)-C(14)	-167.74(19)

C(12)-N(1)-C(15)-C(14)	-26.6(2)
C(13)-C(14)-C(15)-N(1)	39.7(2)
C(2)-C(3)-C(16)-C(21)	60.3(3)
C(4)-C(3)-C(16)-C(21)	-115.2(2)
C(2)-C(3)-C(16)-C(17)	-122.2(2)
C(4)-C(3)-C(16)-C(17)	62.4(3)
C(21)-C(16)-C(17)-C(18)	1.8(3)
C(3)-C(16)-C(17)-C(18)	-175.77(18)
C(16)-C(17)-C(18)-C(19)	0.6(3)
C(17)-C(18)-C(19)-C(20)	-2.0(3)
C(17)-C(18)-C(19)-S(1)	175.80(16)
O(3)-S(1)-C(19)-C(20)	-140.27(18)
O(4)-S(1)-C(19)-C(20)	-9.9(2)
C(22)-S(1)-C(19)-C(20)	104.7(2)
O(3)-S(1)-C(19)-C(18)	41.9(2)
O(4)-S(1)-C(19)-C(18)	172.34(17)
C(22)-S(1)-C(19)-C(18)	-73.1(2)
C(18)-C(19)-C(20)-C(21)	0.9(3)
S(1)-C(19)-C(20)-C(21)	-176.87(16)
C(19)-C(20)-C(21)-C(16)	1.5(3)
C(17)-C(16)-C(21)-C(20)	-2.8(3)
C(3)-C(16)-C(21)-C(20)	174.76(19)
C(3)-C(2)-C(23)-C(24)	28.5(3)
C(1)-C(2)-C(23)-C(24)	-148.5(2)
C(3)-C(2)-C(23)-C(28)	-151.7(2)
C(1)-C(2)-C(23)-C(28)	31.3(3)
C(28)-C(23)-C(24)-C(25)	1.2(3)
C(2)-C(23)-C(24)-C(25)	-179.03(18)
C(23)-C(24)-C(25)-C(26)	-0.6(3)
C(24)-C(25)-C(26)-C(27)	-0.6(3)
C(25)-C(26)-C(27)-C(28)	1.2(3)
C(26)-C(27)-C(28)-C(23)	-0.6(3)
C(24)-C(23)-C(28)-C(27)	-0.6(3)
C(2)-C(23)-C(28)-C(27)	179.63(18)

Symmetry transformations used to generate equivalent atoms:

Table 6. Hydrogen bonds for RFHX-1 [A and deg.].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
C(13)-H(13B)...O(2)#1	0.99	2.64	3.537(3)	150.4
C(14)-H(14B)...O(2)#2	0.99	2.64	3.531(3)	149.1

Symmetry transformations used to generate equivalent atoms:
#1 x,-y+1,z+1/2 #2 x,-y,z+1/2