Supporting Information (Sundaram et. al.)

Characterization of a Brain Permeant Fluorescent Molecule and Visualization of Aβ Parenchymal Plaques, Using Real-time Multiphoton Imaging in Transgenic Mice

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Experimental Section

General Methods.

All reagents were purchased from Sigma-Aldrich unless otherwise stated. 2-fluoroethyl-4-methylbenzene sulfonate was prepared using literature procedure \(^1\). \(^1\)H NMR, proton-decoupled \(^13\)C NMR, and \(^19\)F NMR spectra were recorded on a Varian 400 MHz spectrometer; chemical shifts are reported in δ (ppm) with reference to either TMS or trichlorofluoromethane (CFCl\(_3\)). Mass spectra were obtained from the University of Missouri, Mass Spectrometry facility using nitrobenzyl alcohol (NBA) as matrix and analyzed via HRFab. Purity of the \(4\) was assessed using an HPLC (Waters system 600 equipped with dual λ-detector 2487 set to 280 and 365 nm) with a C-18 reversed-phase column (Vydac, 10μm, 100 Å) using an eluent mixture of acetonitrile and water as a gradient system (50% acetonitrile in water for 5 min; 50-100% acetonitrile in water for 5-25 min, 100% acetonitrile from 25-30 min, and finally 50% acetonitrile in water from 35-50 min, at a flow of 1 mL/min).

Chemical Synthesis.

6-methoxy-2-methyl benzothiazole (1)

N-(2-iodo-4-methoxyphenyl)acetamide was coupled with sodium sulfide, in the presence of copper (I) iodide as a catalyst, to obtain 1 using literature procedure \(^2\) and characterized to confirm its identity and formulation.

(E)-5-(2-(6-methoxybenzo[d]thiazol-2-yl)vinyl)-N,N-dimethylpyridin-2-amine (2)

To a mixture of 6-methoxy-2-methyl benzothiazole (1.0 mmol) and 6-dimethylamino pyridine carbaldehyde (1.0 mmol) in DMSO was added 50 % KOH aqueous solution (10 mL). The resulting mixture was stirred at room temperature for 12 h. After the completion of the reaction, the
reaction mixture was filtered and the resulted solid was washed with water (3 × 5mL). The wet solid was re-dissolved in DCM (15 mL), washed with water (3 × 5 mL), and combined organic extract was dried over the sodium sulfate. The contents were filtered and concentrated under reduced pressure to obtain a yellow solid (2; 0.28 g; 92%; Rf = 0.62, 3:2 hexane: EtOAc) and used for the next step without purification.

![Image of compound 2](image_url)

**1H NMR (400 MHz, CDCl3):** δ 3.15 (s, 6H), 3.88 (s, 3H), 6.55 (d, J = 8.4 Hz, 1H), 7.04 (d, J = 9.2 Hz, 1H), 7.14 (d, J = 16.0 Hz, 1H), 7.29 (t, J = 14.0 Hz, 2H), 7.71 (d, J = 8.4 Hz, 1H), 7.83 (d, J = 8.4 Hz, 1H), 8.29 (s, 1H); **13C NMR (100 MHz, CDCl3):** δ 38.14, 55.79, 104.13, 106.05, 115.31, 118.0, 119.41, 123.02, 134.08, 134.22, 148.93, 159.15, 165.33; HRMS (FAB) m/z calc. for C_{17}H_{18}N_{3}OS: [M+1]^+ 312.1171; found: 312.1167.

(E)-2-(2-(6-(dimethylamino)pyridin-3-yl)vinyl)benzo[d]thiazol-6-ol (3)

The condensed product 2 (0.1 g, 0.3 mmol) was dissolved in dry DCM (5mL) under argon, cooled to -78 °C, stirred for 5 min, and treated with drop-wise addition of BBr₃ (1M in DCM, 0.15 mL, 5.0 mmol). The resulting mixture was slowly brought to room temperature and stirred overnight. Following completion of the reaction (monitored by TLC), the reaction mixture was cooled to 0 °C and quenched with cold satd. sodium bicarbonate solution (5 mL). The reaction mixture was extracted with ethyl acetate (2 × 25 mL), combined extract was washed with water (2 × 10 mL),
dried over anhydrous sodium sulfate, filtered, and the filtrates were evaporated under reduced pressure. The residual red solid was purified by flash chromatography using hexane: EtOAc : MeOH (10: 9: 1) as an eluent mixture to obtain 3 (0.08 g; 86%; Rf = 0.24)

\[ \text{E)-5-(2-(6-(2-fluoroethoxy)benzo[d]thiazol-2-yl)vinyl)-N,N-dimethylpyridin-2-amine (4)} \]

To a solution of alcohol derivative 3 (0.15 g, 0.5 mmol) in DMF (2mL) and 2-fluoroethyltosylate \(^1\) (0.1g, 0.4 mmol) dissolved in DMF (3mL) was added Cs\(_2\)CO\(_3\) (0.20 g, 0.6 mmol). The contents were stirred at 130°C for 5h. Following completion of the reaction (monitored by TLC), the reaction mixture was quenched with ice-cold water and extracted with ethyl acetate (3 x25 mL). The combined organic layer was washed with water (2 x 50 mL), dried over anhydrous sodium sulfate, filtered, and the filtrate evaporated under reduced pressure. The crude residue was purified by

\[ \text{1H NMR (400 MHz, DMSO-}d_6\text{): } \delta 3.03 (s, 6H), 6.65 (d, J = 8.4 Hz, 1H), 6.88 (d, J = 7.6 Hz, 1H), 7.24-7.32 (m, 2H), 7.66 (d, J = 8.4 Hz, 2H), 7.90 (d, J = 7.6 Hz, 1H), 8.29 (s, 1H), 9.82 (s, 1H); \text{13C NMR (100 MHz, DMSO-}d_6\text{): } \delta 38.04, 106.51, 107.08, 116.11, 118.03, 119.58, 123.17, 134.13, 134.96, 135.60, 147.47, 149.36, 155.90, 159.25, 163.97; \text{HRMS (FAB) m/z calc. for C}_{16}\text{H}_{16}\text{N}_{3}\text{OS: [M+1]}^+ 298.1014; \text{found: 298.1015.} \]
PTLC using hexane : EtOAc as an eluent mixture (60 : 40) to obtain 4 (0.096 g; 56% ; bright yellow solid; Rf = 0.42; 3:2, EtOAc-hexane).

\[
\begin{align*}
\text{4} \\
\end{align*}
\]

\(^1\text{H NMR (400 MHz, CDCl}_3\text{): } \delta 3.14 \text{ (s, 6H), 4.20-4.33 (m, 2H), 4.79 (dd, J = 47.6, 8.0 Hz, 2H), 6.51-6.57 (m, 1H), 6.67-6.82 (m, 1H), 7.08 (dd, J = 9.2, 2.6 Hz, 1H), 7.23-7.34 (m, 1H), 7.30-7.34 (m, 1H), 7.70-7.97 (m, 2H), 8.31 (dd, J = 8.8, 2.0 Hz 1H);} \\
\text{\(^{13}\text{C NMR (100 MHz, CDCl}_3\text{): } \delta 38.10, 38.13, 67.63, 67.84, 81.01, 82.70, 105.32, 106.05, 115.67, 117.86, 119.34, 123.12, 134.23, 134.33, 148.98, 156.36, 159.17, 165.74;} \\
\text{\(^{19}\text{F NMR (282 MHz, CFCl}_3\text{): -224 ppm;} \\
\text{HRMS (FAB) m/z calc. for C}_{18}\text{H}_{19}\text{FN}_{3}\text{OS: [M+1]}^+ 344.1232; found: 343.1230.}
\]

**X-ray Crystallography.** Crystals suitable for X-ray crystallography were grown by vapor diffusion method by dissolving 4 (4 mg) in methylene chloride and ethyl acetate (1mL; 95:5) in an inner vial and pentane as anti-solvent (1.5 mL) in the outer vial at room temperature over a couple of days. A single crystal with approximate dimensions 0.446 x 0.204 x 0.089 mm\(^3\) was mounted on a MiTegen loop in a random orientation. Preliminary examination and data collection were performed using a Bruker Kappa Apex II (Charge Coupled Device (CCD) Detector system) single crystal X-Ray diffractometer, equipped with an Oxford Cryostream LT device. All data were collected using graphite monochromated Mo K\(\alpha\) radiation (\(\lambda = 0.71073\) Å) from a fine focus sealed
tube X-Ray source. Preliminary unit cell constants were determined with a set of 36 narrow frame
scans. Collected data set consisted of a combination of \( \varpi \) and \( \phi \) scans with a scan width of 0.5°
and counting time of 20 seconds/frame at a crystal to detector distance of 4.0 cm. The collected
frames were integrated using an orientation matrix determined from the narrow frame scans.
Apex II and SAINT software packages \(^3\) were used for data collection and data integration.
Analysis of the integrated data did not show any decay. Final cell constants were determined by
global refinement of xyz centroids of 9700 reflections from the complete data set. Collected data
were corrected for systematic errors using SADABS \(^3\) based on the Laue symmetry using
equivalent reflections. Structure solution and refinement were carried out using the SHELXTL-
PLUS software package \(^3\). The structure was solved by direct methods and refined successfully in
the space group, \( P \overline{1} \). Full matrix least-squares refinement was carried out by minimizing \(*w(F_0^2 -
F_c^2)^2*\). The non-hydrogen atoms were refined anisotropically to convergence. All hydrogen atoms
were treated using appropriate riding model (AFIX m3). The F atom was disordered over two
positions and was refined to occupancies of 94:6% and was refined with geometrical restraints
and displacement constraints. A fragment search of the Cambridge database \(^4\) indicated 31
structures with the fragment C3 to C12, N1, S1. The bond angles and distances for this fragment
compare well with the mean distance angles calculated from the data base, using Mogul (Mogul
1.6, Cambridge Structural Data Center) \(^5\). Analysis of intermolecular interactions shows a weak \( \pi-\)
\( \pi \) interaction and hydrogen bonding. Weak \( \pi-\pi \) interaction is also observed between the \( \pi-\)
systems of the phenyl ring (C3 to C8, plane #1) of the benzothiazole ring and the six membered
ring C12-C16, N2 (plane #2) with a centroid to centroid distance of 3.826Å [plane #1 and #2 (2-x,
2-y, 1-z)] (Olex2, V1.2). Weak hydrogen bonding between nitrogen (N1) and the CH hydrogen
(H1b) [C1-H1b...N2(1, x, 2-y, 1-z) =2.59 Å, 172.1°], F atom F1 with aromatic hydrogen H4 from the phenyl ring of the benzothiazole moiety [C4-H4...F1(1+x, y, z) =2.55 Å, 140.2°] and the oxygen atom O1 with aromatic hydrogen H5 from the phenyl ring of the benzothiazole moiety [C5-H5...O1(1+x, y, z) =2.62 Å, 143.4°] are also observed in the crystal structure. The ORTEP drawing showing the crystallographic numbering scheme for 4 is given in Figure 1. A packing plot showing the hydrogen bonding interactions is shown in Figure 2.

Bioassays.

Preparation of Aβ fibrils

Commercially available amyloid peptide Aβ₁₋₄₂ (433 µg) was gently dissolved in PBS (1 mL, pH 7.4, 100 µM). The solution was incubated for 36-48 h at 37°C with continuous gentle shaking to avoid gel formation at the meniscus. The aggregated peptide suspension was stored at -80°C until needed (the suspension did not show any noticeable change in properties for at least 8 weeks).

Binding of 4 with Aβ fibrils

*In vitro* binding assays to preformed fibrils were performed using literature procedures. Prior to binding assays, the Aβ fibril stock solution was thawed, diluted with PBS to the final concentration of Aβ fibrils (1µM), and incubated with increasing concentrations of the fluorescent molecule 4 for 30 min. Following excitation at 410nm, fluorescence spectrum of 4 recorded in PBS containing 5% ethanol showed a broad emission peak 450-540nm with $E_{\text{max}}$ at 503nm. Upon incubation with preformed of Aβ₁₋₄₂ aggregates, the peak at 503nm shifted slightly ($E_{\text{max}}$ 485 nm) and showed remarkable enhancement in fluorescence (*Figure 3*), indicating binding to Aβ aggregates, similar to enhancement in fluorescence of thioflavin-T in PBS (a positive control; data not shown). Additionally, it is also noteworthy that no fluorescence was observed using Aβ...
aggregates alone in PBS upon excitation at 410 nm (a negative control). All measurements were made in triplicates; fluorescence of either 4 alone or fibrils at a given concentration was subtracted. The data were fit to a single site model using GraphPad Prism, version 4.03 (GraphPad Software, San Diego, California, USA) to obtain the binding constant 59±7nM (Figure 4).

**Histochemical Staining of Mouse Brain Tissues**

Mice, 24 month old APP+/−/PS1+/− and age-matched control BL/6 (WT), were sacrificed and their brains removed, fixed in 4% formaldehyde, and stored in 30% sucrose for 3 days. Serial brain tissue sections (50 µm) were cut in the coronal plane on a freezing sliding microtome and stored at -20°C. Tissue was stained as free-floating sections. Prior to staining, tissue sections were permeabilized with PBS-Triton-X-100 (0.25%) for 30 minutes, and then blocked with 1% non-fat dry milk in PBS-Triton-X-100 for 60 minutes. Tissue sections were incubated with fluorescent molecule 4 (1 µM dissolved in 1% ethanol in PBS) for 60 minutes, and washed 3-times with PBS-Triton-X-100. Thereafter, tissue sections were incubated with mouse-anti-Aβ (mHJ3.4)8 that was directly conjugated to Alexa 568 for 90 minutes and washed 3-times with PBS-Triton-X-100. Tissue sections were mounted onto SuperFrost Plus slides, dried overnight at room temperature, and then sealed with Fluoromount G (Southern Biotech, Birmingham, AL). Finally, stained sections were imaged using a Nikon Eclipse E800 epifluorescence microscope.

**Histological Staining of Human Brain Tissue**

Postmortem brain tissues from autopsy-confirmed AD patients and their approximate age-matched healthy controls were obtained through the Knight Alzheimer’s Disease Research Center (ADRC) at our institution and processed according to a protocol approved by institutional ADRC executive committees11. Formalin-fixed, paraffin embedded tissue sections were obtained from AD brains and age-matched neurological and neuropathological controls. Brain tissue samples
were either fixed in 4% formaldehyde, and stored in 30% sucrose for 3 days and sliced (10 µm) a freezing sliding microtome; or tissue was fixed in 10% neutral buffered formal saline, embedded in paraffin wax, and cut at 10 µm. Tissue sections were incubated with either a fluorescent molecule 4 (10 µM) or treated with anti-Aβ (10D5, dilution: 1:10,000; Eli Lilly, Indianapolis, IN) or thioflavin-S. Sections were visualized, processed, and analyzed using a Pascal confocal microscope.

**Multiphoton Imaging**

17-month-old APP⁺⁻/PS1⁺⁻ mice (harboring dense amyloid plaque accumulation) were imaged using intravital multiphoton microscopy to assess pharmacokinetic profiles of the compound. Using procedures approved by the Animal Studies Committee at Washington University, thinned-skull cranial windows were prepared on the day prior to imaging, as previously described ¹²,¹³. Briefly, mice were anesthetized under volatile isoflurane (2% induction, 1.5% maintenance), the scalp and periosteum was removed to expose the skull. A speed drill and microsurgical blade (Surgistar) were used to thin the skull until the skull window (1.5 mM in diameter) was transparent and displayed flexibility (approximately 10-20 µm thick). Pial vasculature was clearly visualized through the window. The day after window preparation, mice were positioned so that the cranial window was directly under the objective lens on a multiphoton-photon microscope [LSM 510 META NLO system (Carl Zeiss Inc.) with a Chameleon Ti: Sapphire laser (Coherent Inc.)]. Prior to imaging, mice were intravenously injected with dextran-Texas Red conjugate to mark blood vessels (33mg/kg dissolved in PBS) and 4 (2 mg/kg dissolved in 20% DMSO in 80% propylene glycol) ¹⁴. Three-dimensional volumes were acquired (by collecting a stack of x-y sections starting at the surface of the thinned skull to 100 microns deep into the cortex) for assessing pharmacokinetics of 4 at high resolution in brains of live mice.
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Figure Legends.

Figure 1. ORTEP drawing showing the crystallographic numbering scheme for 4.

Figure 2. A packing plot for 4 showing intermolecular hydrogen bonding interactions.

Figure 3. Fluorescence of 4 in either presence or absence of Aβ1-42 aggregates in PBS:EtOH (95:5).

Figure 4. Binding of 4 with preformed Aβ1-42 aggregates. For the binding assay, the 4 was incubated in increasing concentrations with a fixed concentration of Aβ1-42 aggregates (1µM) at 37°C for 30 min in PBS. Fluorescence of 4 alone and fibrils at a given concentration was subtracted. All measurements were made in triplicates; error bars represent SD. The data were fitted into a single site model using GraphPad Prism, version 4.03 (GraphPad Software, San Diego, Ca, USA) and the binding constant was found to be 59±7nM.

Figure 5. Staining of brain tissue cortex sections (50 µm) from 9 month old WT mice using 4 (1 µM). Magnification: 20×. Diffuse nonspecific specks of green color indicate autofluorescent spots. Similar results were obtained in more than 3 independent experiments.

Figure 6. Staining of brain tissue cortex sections from human postmortem brain of a cognitively and neuropathologically normal subject using 4 (10 µM). Magnification: 10×. The fluorescent molecule 4 does not show any pathology (neither Aβ amyloid plaques nor vascular amyloid) in a
clinically and neuropathologically normal brain. Similar results were obtained in more than 3 independent cases.
Figure 3

Fluorescence (a.u.)

Wavelength (nm)

- no fibrils
- with fibrils

**Figure 3**
Analytical Data for Compounds.

NMR Spectral Data

$^1$H spectrum of compound 2
$^{13}$C NMR Spectrum of compound 2

$^1$H spectrum of compound 3

$^{13}$C NMR Spectrum of compound 3
\begin{align*}
\text{\textsuperscript{13}C NMR Spectrum of compound 4} \\
\text{\textsuperscript{1}H spectrum of compound 4}
\end{align*}
$^{19}$F NMR Spectrum for 4

HPLC Chromatogram for 4
## Crystal Data Tables (4)

**Table 1.** Crystal data and structure refinement for 4.

| Property                                  | Value                                    |
|-------------------------------------------|------------------------------------------|
| Identification code                       | v813/rt/VS-182                           |
| Empirical formula                        | C\textsubscript{18} H\textsubscript{18} F N\textsubscript{3} O S |
| Formula weight                            | 343.41                                   |
| Temperature                               | 300(2) K                                 |
| Wavelength                                | 0.71073 Å                                |
| Crystal system                            | Triclinic                                |
| Space group                               | P -1                                     |
| Unit cell dimensions                      | a = 6.3565(2) Å, b = 9.5072(4) Å, c = 14.1841(6) Å |
|                                           | α = 77.295(2)°, β = 77.987(2)°, γ = 87.843(2)° |
| Volume                                    | 817.85(6) Å\textsuperscript{3}           |
| Z                                         | 2                                        |
| Density (calculated)                      | 1.395 Mg/m\textsuperscript{3}           |
| Absorption coefficient                    | 0.218 mm\textsuperscript{-1}            |
| F(000)                                    | 360                                      |
| Crystal size                              | 0.446 x 0.204 x 0.089 mm\textsuperscript{3} |
| Theta range for data collection           | 2.196 to 28.879°.                       |
| Index ranges                              | -8≤h≤8, -12≤k≤12, -19≤l≤19               |
| Reflections collected                     | 31827                                    |
| Independent reflections                   | 4298 [R(int) = 0.0328]                   |
| Completeness to theta = 25.242°           | 99.9 %                                   |
| Absorption correction                     | Semi-empirical from equivalents         |
| Max. and min. transmission                | 0.9808 and 0.9089                        |
| Refinement method                         | Full-matrix least-squares on F\textsuperscript{2} |
| Data / restraints / parameters            | 4298 / 13 / 223                         |
Goodness-of-fit on $F^2$ 1.025
Final R indices [I>2sigma(I)] R1 = 0.0411, wR2 = 0.1050
R indices (all data) R1 = 0.0613, wR2 = 0.1192
Extinction coefficient n/a
Largest diff. peak and hole 0.255 and -0.279 e.Å$^{-3}$
Table 2. Atomic coordinates (x $10^4$) and equivalent isotropic displacement parameters ($Å^2x10^3$) for 4. U(eq) is defined as one third of the trace of the orthogonalized $U_{ij}$ tensor.

|   | x   | y   | z   | U(eq) |
|---|-----|-----|-----|-------|
| S(1) | 6548(1) | 8909(1) | 5784(1) | 42(1) |
| F(1) | -1126(2) | 11729(2) | 9980(1) | 92(1) |
| F(1') | 170(30) | 13780(20) | 10095(16) | 92(1) |
| O(1) | 2550(2) | 11621(1) | 8381(1) | 51(1) |
| N(1) | 9719(2) | 8921(2) | 6675(1) | 42(1) |
| N(2) | 11827(2) | 5337(2) | 2526(1) | 47(1) |
| N(3) | 14854(3) | 4085(2) | 2000(1) | 56(1) |
| C(1) | 348(3) | 12804(3) | 9510(2) | 66(1) |
| C(2) | 2523(3) | 12203(2) | 9221(1) | 51(1) |
| C(3) | 4397(2) | 10964(2) | 8002(1) | 40(1) |
| C(4) | 6255(3) | 10861(2) | 8396(1) | 44(1) |
| C(5) | 8051(2) | 10201(2) | 7978(1) | 45(1) |
| C(6) | 8030(2) | 9631(2) | 7159(1) | 38(1) |
| C(7) | 6146(2) | 9735(2) | 6777(1) | 36(1) |
| C(8) | 4321(2) | 10403(2) | 7191(1) | 40(1) |
| C(9) | 9184(2) | 8486(2) | 5955(1) | 39(1) |
| C(10) | 10605(3) | 7702(2) | 5324(1) | 42(1) |
| C(11) | 10135(2) | 7255(2) | 4568(1) | 42(1) |
| C(12) | 11483(2) | 6442(2) | 3927(1) | 39(1) |
| C(13) | 13550(3) | 5953(2) | 4026(1) | 45(1) |
| C(14) | 14701(3) | 5176(2) | 3402(1) | 46(1) |
| C(15) | 13793(3) | 4863(2) | 2647(1) | 42(1) |
| C(16) | 10763(3) | 6096(2) | 3154(1) | 46(1) |
| C(17) | 16991(3) | 3528(2) | 2069(2) | 64(1) |
| C(18) | 13808(4) | 3713(2) | 1278(1) | 63(1) |
### Table 3. Bond lengths [Å] and angles [°] for 4.

| Bond  | Length [Å] |
|-------|------------|
| S(1)-C(7) | 1.7280(15) |
| S(1)-C(9) | 1.7618(15) |
| F(1)-C(1) | 1.371(3) |
| F(1')-C(1) | 1.362(6) |
| O(1)-C(3) | 1.3742(18) |
| O(1)-C(2) | 1.415(2) |
| N(1)-C(9) | 1.291(2) |
| N(1)-C(6) | 1.3870(19) |
| N(2)-C(16) | 1.329(2) |
| N(2)-C(15) | 1.345(2) |
| N(3)-C(15) | 1.359(2) |
| N(3)-C(18) | 1.441(3) |
| N(3)-C(17) | 1.454(2) |
| C(1)-C(2) | 1.487(3) |
| C(1)-H(1A) | 0.9700 |
| C(1)-H(1B) | 0.9700 |
| C(2)-H(2A) | 0.9700 |
| C(2)-H(2B) | 0.9700 |
| C(3)-C(8) | 1.379(2) |
| C(3)-C(4) | 1.400(2) |
| C(4)-C(5) | 1.370(2) |
| C(4)-H(4) | 0.9300 |
| C(5)-C(6) | 1.387(2) |
| C(5)-H(5) | 0.9300 |
| C(6)-C(7) | 1.406(2) |
| C(7)-C(8) | 1.386(2) |
| C(8)-H(8) | 0.9300 |
| C(9)-C(10) | 1.443(2) |
| C(10)-C(11) | 1.327(2) |
| C(10)-H(10) | 0.9300 |
| C(11)-C(12) | 1.447(2) |
| C(11)-H(11) | 0.9300 |
| C(12)-C(16) | 1.379(2) |
| C(12)-C(13) | 1.402(2) |
| Bond          | Distance (Å) |
|--------------|-------------|
| C(13)-C(14)  | 1.357(2)    |
| C(13)-H(13)  | 0.9300      |
| C(14)-C(15)  | 1.407(2)    |
| C(14)-H(14)  | 0.9300      |
| C(16)-H(16)  | 0.9300      |
| C(17)-H(17A) | 0.9600      |
| C(17)-H(17B) | 0.9600      |
| C(17)-H(17C) | 0.9600      |
| C(18)-H(18A) | 0.9600      |
| C(18)-H(18B) | 0.9600      |
| C(18)-H(18C) | 0.9600      |
| C(7)-S(1)-C(9)| 89.11(7)   |
| C(3)-O(1)-C(2)| 118.21(12) |
| C(9)-N(1)-C(6)| 111.00(13) |
| C(16)-N(2)-C(15)| 117.53(15) |
| C(15)-N(3)-C(18)| 120.15(16) |
| C(15)-N(3)-C(17)| 120.83(16) |
| C(18)-N(3)-C(17)| 118.87(16) |
| F(1')-C(1)-C(2)| 117.0(10)  |
| F(1)-C(1)-C(2)| 111.21(18) |
| F(1)-C(1)-H(1A)| 109.4      |
| C(2)-C(1)-H(1A)| 109.4      |
| F(1)-C(1)-H(1B)| 109.4      |
| C(2)-C(1)-H(1B)| 109.4      |
| H(1A)-C(1)-H(1B)| 108.0      |
| O(1)-C(2)-C(1)| 108.12(15) |
| O(1)-C(2)-H(2A)| 110.1      |
| C(1)-C(2)-H(2A)| 110.1      |
| O(1)-C(2)-H(2B)| 110.1      |
| C(1)-C(2)-H(2B)| 110.1      |
| H(2A)-C(2)-H(2B)| 108.4      |
| O(1)-C(3)-C(8)| 115.61(13) |
| O(1)-C(3)-C(4)| 123.18(14) |
| C(8)-C(3)-C(4)| 121.21(14) |
| C(5)-C(4)-C(3)| 120.30(15) |
C(5)-C(4)-H(4)  119.8
C(3)-C(4)-H(4)  119.8
C(4)-C(5)-C(6)  119.91(14)
C(4)-C(5)-H(5)  120.0
C(6)-C(5)-H(5)  120.0
N(1)-C(6)-C(5)  125.76(14)
N(1)-C(6)-C(7)  115.17(14)
C(5)-C(6)-C(7)  119.06(14)
C(8)-C(7)-C(6)  121.64(14)
C(8)-C(7)-S(1)  129.08(12)
C(6)-C(7)-S(1)  109.27(11)
C(3)-C(8)-C(7)  117.88(14)
C(3)-C(8)-H(8)  121.1
C(7)-C(8)-H(8)  121.1
N(1)-C(9)-C(10)  123.60(14)
N(1)-C(9)-S(1)  115.44(12)
C(10)-C(9)-S(1)  120.96(12)
C(11)-C(10)-C(9)  125.61(15)
C(11)-C(10)-H(10)  117.2
C(9)-C(10)-H(10)  117.2
C(10)-C(11)-C(12)  127.72(15)
C(10)-C(11)-H(11)  116.1
C(12)-C(11)-H(11)  116.1
C(16)-C(12)-C(13)  115.18(15)
C(16)-C(12)-C(11)  120.28(14)
C(13)-C(12)-C(11)  124.54(15)
C(14)-C(13)-C(12)  120.97(16)
C(14)-C(13)-H(13)  119.5
C(12)-C(13)-H(13)  119.5
C(13)-C(14)-C(15)  119.09(15)
C(13)-C(14)-H(14)  120.5
C(15)-C(14)-H(14)  120.5
N(2)-C(15)-N(3)  116.70(16)
N(2)-C(15)-C(14)  121.20(15)
N(3)-C(15)-C(14)  122.10(15)
N(2)-C(16)-C(12)  126.01(15)
| Bond                        | Angle  |
|-----------------------------|--------|
| N(2)-C(16)-H(16)            | 117.0  |
| C(12)-C(16)-H(16)           | 117.0  |
| N(3)-C(17)-H(17A)           | 109.5  |
| N(3)-C(17)-H(17B)           | 109.5  |
| H(17A)-C(17)-H(17B)         | 109.5  |
| N(3)-C(17)-H(17C)           | 109.5  |
| H(17A)-C(17)-H(17C)         | 109.5  |
| H(17B)-C(17)-H(17C)         | 109.5  |
| N(3)-C(18)-H(18A)           | 109.5  |
| N(3)-C(18)-H(18B)           | 109.5  |
| H(18A)-C(18)-H(18B)         | 109.5  |
| N(3)-C(18)-H(18C)           | 109.5  |
| H(18A)-C(18)-H(18C)         | 109.5  |
| H(18B)-C(18)-H(18C)         | 109.5  |
Table 4. Anisotropic displacement parameters (Å² x 10³) for 4. The anisotropic displacement factor exponent takes the form: 
\[-2\pi^2 [ h^2 a^* U_{11} + ... + 2hk a^* b^* U_{12} ]\]

|    | U₁₁  | U₂₂  | U₃₃  | U₁₂  | U₁₃  | U₂₃  |
|----|------|------|------|------|------|------|
| S(1)| 38(1)| 51(1)| 42(1)| -20(1)| -13(1)| 6(1) |
| F(1)| 62(1)| 139(2)| 77(1)| -40(1)| 2(1)  | -9(1)|
| F(1')| 62(1)| 139(2)| 77(1)| -40(1)| 2(1)  | -9(1)|
O(1)| 43(1)| 70(1)| 48(1)| -32(1)| -12(1)| 12(1)|
N(1)| 37(1)| 49(1)| 45(1)| -15(1)| -12(1)| 6(1)|
N(2)| 51(1)| 50(1)| 47(1)| -18(1)| -18(1)| 7(1)|
N(3)| 62(1)| 61(1)| 51(1)| -26(1)| -11(1)| 13(1)|
C(1)| 64(1)| 88(2)| 56(1)| -38(1)| -13(1)| 15(1)|
C(2)| 54(1)| 62(1)| 44(1)| -25(1)| -11(1)| 4(1)|
C(3)| 37(1)| 45(1)| 40(1)| -13(1)| -7(1) | 1(1)|
C(4)| 44(1)| 54(1)| 41(1)| -20(1)| -12(1)| -2(1)|
C(5)| 39(1)| 57(1)| 46(1)| -18(1)| -16(1)| 1(1)|
C(6)| 35(1)| 40(1)| 38(1)| -9(1)  | -9(1) | 0(1)|
C(7)| 37(1)| 37(1)| 34(1)| -10(1)| -9(1) | -1(1)|
C(8)| 35(1)| 47(1)| 42(1)| -15(1)| -12(1)| 4(1)|
C(9)| 38(1)| 37(1)| 40(1)| -7(1)  | -8(1) | 3(1)|
C(10)| 40(1)| 43(1)| 45(1)| -12(1)| -9(1) | 8(1)|
C(11)| 40(1)| 41(1)| 46(1)| -9(1)  | -9(1) | 5(1)|
C(12)| 41(1)| 36(1)| 40(1)| -9(1)  | -9(1) | 3(1)|
C(13)| 46(1)| 49(1)| 47(1)| -17(1)| -17(1)| 7(1)|
C(14)| 40(1)| 52(1)| 50(1)| -18(1)| -14(1)| 10(1)|
C(15)| 48(1)| 38(1)| 40(1)| -10(1)| -6(1) | 2(1)|
C(16)| 43(1)| 49(1)| 50(1)| -16(1)| -16(1)| 7(1)|
C(17)| 54(1)| 67(1)| 70(1)| -28(1)| 3(1)  | 12(1)|
C(18)| 86(1)| 61(1)| 46(1)| -21(1)| -11(1)| 1(1)|
Table 5. Hydrogen coordinates ( x $10^4$) and isotropic displacement parameters (Å$^2 x 10^3$) for 4.

| H  | x     | y     | z     | U(eq) |
|----|-------|-------|-------|-------|
| H(1A) | 410   | 13453 | 9944  | 99    |
| H(1B) | -103  | 13354 | 8925  | 99    |
| H(2A) | 3606  | 12957 | 9067  | 61    |
| H(2B) | 2833  | 11456 | 9759  | 61    |
| H(4)  | 6271  | 11242 | 8945  | 53    |
| H(5)  | 9282  | 10135 | 8242  | 54    |
| H(8)  | 3084  | 10471 | 6931  | 48    |
| H(10) | 11968 | 7491  | 5458  | 51    |
| H(11) | 8778  | 7492  | 4435  | 51    |
| H(13) | 14142 | 6164  | 4526  | 55    |
| H(14) | 16072 | 4855  | 3473  | 55    |
| H(16) | 9408  | 6425  | 3062  | 55    |
| H(17A) | 17972 | 4317  | 1965  | 96    |
| H(17B) | 17463 | 2972  | 1576  | 96    |
| H(17C) | 16948 | 2926  | 2713  | 96    |
| H(18A) | 12468 | 3232  | 1605  | 95    |
| H(18B) | 14717 | 3084  | 928   | 95    |
| H(18C) | 13543 | 4574  | 820   | 95    |
Table 6. Torsion angles [°] for 4.

| Bond                        | Torsion Angle [°]   |
|-----------------------------|--------------------|
| C(3)-O(1)-C(2)-C(1)        | -178.07(16)        |
| F(1')-C(1)-C(2)-O(1)       | -160.7(12)         |
| F(1)-C(1)-C(2)-O(1)        | 76.1(2)            |
| C(2)-O(1)-C(3)-C(8)        | 179.28(15)         |
| C(2)-O(1)-C(3)-C(4)        | -0.9(2)            |
| O(1)-C(3)-C(4)-C(5)        | -179.46(15)        |
| C(8)-C(3)-C(4)-C(5)        | 0.3(3)             |
| C(3)-C(4)-C(5)-C(6)        | 0.0(3)             |
| C(9)-N(1)-C(6)-C(7)        | 178.37(15)         |
| C(9)-N(1)-C(6)-C(7)        | -0.3(2)            |
| C(4)-C(5)-C(6)-N(1)        | -179.03(15)        |
| C(4)-C(5)-C(6)-C(7)        | -0.4(2)            |
| N(1)-C(6)-C(7)-C(8)        | 179.36(14)         |
| C(5)-C(6)-C(7)-C(8)        | 0.6(2)             |
| N(1)-C(6)-C(7)-S(1)        | 0.06(17)           |
| C(5)-C(6)-C(7)-S(1)        | -178.70(12)        |
| C(9)-S(1)-C(7)-C(8)        | 179.66(14)         |
| C(9)-S(1)-C(7)-C(8)        | 0.13(12)           |
| O(1)-C(3)-C(8)-C(7)        | 179.83(12)         |
| C(4)-C(3)-C(8)-C(7)        | -0.1(2)            |
| C(6)-C(7)-C(8)-C(3)        | -0.3(2)            |
| S(1)-C(7)-C(8)-C(3)        | 178.83(12)         |
| C(6)-N(1)-C(9)-C(10)       | -179.37(14)        |
| C(6)-N(1)-C(9)-S(1)        | 0.40(18)           |
| C(7)-S(1)-C(9)-N(1)        | -0.32(13)          |
| C(7)-S(1)-C(9)-C(10)       | 179.46(14)         |
| N(1)-C(9)-C(10)-C(11)      | -178.90(16)        |
| S(1)-C(9)-C(10)-C(11)      | 1.3(2)             |
| C(9)-C(10)-C(11)-C(12)     | -178.64(15)        |
| C(10)-C(11)-C(12)-C(16)    | -178.17(17)        |
| C(10)-C(11)-C(12)-C(13)    | 1.7(3)             |
| C(16)-C(12)-C(13)-C(14)    | -1.1(2)            |
| C(11)-C(12)-C(13)-C(14)    | 178.97(16)         |
| C(12)-C(13)-C(14)-C(15)    | 0.0(3)             |
| Bond                        | Angle         |
|-----------------------------|---------------|
| C(16)-N(2)-C(15)-N(3)      | 179.83(15)    |
| C(16)-N(2)-C(15)-C(14)     | -0.8(2)       |
| C(18)-N(3)-C(15)-N(2)      | -4.6(2)       |
| C(17)-N(3)-C(15)-N(2)      | 179.87(16)    |
| C(18)-N(3)-C(15)-C(14)     | 176.07(17)    |
| C(17)-N(3)-C(15)-C(14)     | 0.6(3)        |
| C(13)-C(14)-C(15)-N(2)     | 1.0(3)        |
| C(13)-C(14)-C(15)-N(3)     | -179.72(16)   |
| C(15)-N(2)-C(16)-C(12)     | -0.4(3)       |
| C(13)-C(12)-C(16)-N(2)     | 1.3(3)        |
| C(11)-C(12)-C(16)-N(2)     | -178.75(16)   |
Table 7. Hydrogen bonds for 4 [Å and °].

| D-H...A          | d(D-H) | d(H...A) | d(D...A)    | <(DHA) |
|------------------|--------|----------|-------------|--------|
| C(1)-H(1B)...N(2)#1 | 0.97   | 2.59     | 3.550(3)    | 172.1  |
| C(4)-H(4)...F(1)#2 | 0.93   | 2.55     | 3.313(2)    | 140.2  |
| C(5)-H(5)...O(1)#2 | 0.93   | 2.62     | 3.4125(19)  | 143.4  |
| C(17)-H(17B)...F(1')#3 | 0.96   | 2.42     | 3.059(19)   | 124.0  |

Symmetry transformations used to generate equivalent atoms:

#1 -x+1,-y+2,-z+1  #2 x+1,y,z  #3 x+2,y-1,z-1