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Supplementary Information for

A small-molecule factor B inhibitor for the treatment of complement-mediated diseases

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Supplementary Information Text

Supporting Materials and Methods

Reagents and antibodies. Cobra venom factor (CVF) was purchased from Quidel Corp., San Diego, USA. Alkaline phosphatase-conjugated or unlabelled anti-C9 neo-epitope antibody (clone aE11) for detection of MAC formation was custom ordered from Diatec Monoclonals AS (Oslo, Norway) and Thermo Fisher. Anti-human C3 was purchased from MP Biomedicals LLC, Santa Ana, CA. For Western blot detection of mouse Ba and C3d+iC3b, a polyclonal anti-human factor B (Quidel, A311) and polyclonal goat anti-mouse C3d/iC3b (R&D Systems; AF2655) were used, respectively. The anti-human C5 antibody was produced internally by phage display technology. For ELISA assays, Black MaxiSorb™ plates were purchased from NUNC/Thermo Scientific (#460518). Zymosan A was from Sigma (#Z4250), horseradish peroxidase (HRP)-conjugated goat anti-rat IgG was from Southern Biotech and Quanta blue was from Pierce Thermo Fisher.

Expression and purification of recombinant human and mouse FB. The DNA coding for the catalytic domain (D470–L764) of human FB (UniProt P00751) was amplified by PCR from an in-house cDNA library. The PCR product was cloned into pMT-BIP resulting in a plasmid that contains the BIP leader sequence at the N-terminus. The Drosophila SL3 cell line, grown in Sf-900 II medium containing 1% FCS (Invitrogen), was used for transfection and generation of stable expression pools. For large scale production, cells were grown in 3 L shake flasks (Corning), each containing 1 L culture at a cell density of 2 x 10^6 cells/mL in Sf-900 II medium (containing 1% FCS and 250 µg/mL hygromycin B), and induced 24 h post-seeding by addition of 200 µM $ZnCl_2$ and 10 µM $CdCl_2$ for 72 h. The supernatant was harvested after centrifugation (2,400 g for 20 minutes at room temperature) and filtered (0.22 µm filter, Millipore). Bulk protein was precipitated with PEG (13% (w/v) PEG-3350 final concentration, 6 h at 4 ºC, spun down at 17,000 g for 20 minutes) and the supernatant dialyzed (4 ºC, 20 mM Tris, pH 6.8). Purification was accomplished by ion-exchange chromatography (Source 15S column, GE Healthcare) using a linear gradient of 0-0.4 M NaCl followed by size-exclusion chromatography (SEC) (Superdex 75 HiLoad16/60, 50 mM Tris, pH 6.8, 100 mM NaCl). Fractions containing FB protein were pooled and concentrated to 48 mg/mL. LC/MS analysis confirmed the expected MW of 33646.5 Da.

The DNA coding for mouse FB (P27-L764) with a C-terminal Strep-tag (WSHPQFEK) was cloned into pMT-BIP. A stable expression pool of SL3 cells was generated and large scale production was done in an analogous way to human FB. Mouse FB was purified from the culture supernatant by addition of Strep-Tactin Sepharose (10 mL/L of supernatant). After 16 h incubation at 4 ºC, the Sepharose was collected by centrifugation (10 minutes at 2,500 g) and the protein eluted with 50 mM Tris, pH 8.0, 150 mM NaCl and 2.5 mM desthiobiotin. Mouse FB was further purified by SEC (Superdex 75 HiLoad16/60, 20 mM

Tris, pH 7.5, and 100 mM NaCl) and concentrated to 1 mg/mL. LC/MS analysis confirmed the expected MW of 87015 Da after deglycosylation.

Purification of C3. C3 was purified from frozen human serum (Swiss Red Cross Bloodbank, Basel). Proteins, lipids and debris were precipitated from 400 mL of serum by slow addition of 21 mL 60% (w/v) PEG3350 in water (final concentration 3% (w/v) PEG3350). The mixture was stirred on ice for 30 minutes and centrifuged at 16,000 g for 10 minutes at 4 °C. The supernatant was collected and C3 was precipitated by further addition of 90 mL 60% (w/v) PEG3350 solution (to a total concentration of 13% (w/v) PEG). After stirring on ice for 30 minutes, the C3 containing precipitate was collected by centrifugation as above. The resulting pellet was dissolved in 0.5 L of buffer A (3 mM potassium phosphate, 6.5 mM EDTA, 6.5 mM benzamidine, 33 mM ε-aminocapronic acid, 50 mM NaCl, pH 7.3) and applied to an anion exchange chromatography (Source Q15; 16x100 mm equilibrated in buffer A). Proteins were eluted with a linear gradient of 0-0.4 M NaCl in buffer A. C3 containing fractions were immediately diluted 5-10-fold in 20 mM potassium phosphate pH 6.0 (buffer B) and applied to a cation exchange chromatography (Source S15; 16x100 mm equilibrated with buffer B). C3 was eluted with a linear gradient of 0-0.25 M NaCl in buffer B. During this step, native (thioester-intact) C3 was separated from water-hydrolyzed C3 (C3(H_2O)). Native C3 containing fractions were identified by SDS-PAGE as above, concentrated by ultrafiltration (10 kDa cutoff, Millipore) and applied to a size exclusion chromatography (26/60 Sephacryl 300, equilibrated with 20 mM potassium phosphate, pH 6.0, 500 mM NaCl). C3 containing fractions were pooled and frozen in liquid nitrogen.

FB inhibition assays. CVF (1 μM), FB (1 μM) and FD (300 nM) were incubated in PBS at pH 7.4, containing 10 mM $MgCl_2$, and 0.05% (w/v) CHAPS (assay buffer) for 3 h at room temperature to allow all FB to be activated by FD cleavage into Ba and the enzymatically active CVF:Bb complex (C3 convertase). The complete hydrolysis of FB was confirmed by SDS-PAGE analysis. Human CVF:Bb complex (3 nM concentration) was pre-incubated with varying compound concentrations for 1 h at room temperature in assay buffer. The enzymatic reaction was started by addition of C3 diluted in assay buffer to a final concentration of 1 μM. After 1 h incubation at room temperature, the enzyme reaction was stopped by addition of a protease inhibitor cocktail (Roche Complete Inhibitor tablets). Generation of C3a was quantified by an enzyme-linked-immunosorbent assay (ELISA). Aliquots (3 μL) of reaction samples were pipetted into 384-well high capacity protein binding plates (NUNC Maxisorp™) pre-filled with 97 μL/well of 100 mM sodium carbonate buffer, pH 9.0, containing 1 M NaCl. After overnight incubation at 4 °C, assay plates were washed with PBS, pH 7.4, containing 0.05% (v/v) Tween 20 (washing buffer). Remaining free binding capacity was saturated by the addition of Starting Block T20 (PIERCE, #37539) for 5 minutes at room temperature, and assay plates were then washed

with washing buffer. Anti-C3a neo-epitope antibody (#C7850-13G, USBIOlogical, Swampscott, MA, USA) was added to each well (diluted in washing buffer; 0.2 µg/well), followed by incubation for 60 minutes at room temperature and removal of excess antibody by washing with washing buffer. Goat anti-mouse antibody labeled with HRP (#A0168, Sigma, Buchs, Switzerland) (0.2 µg/well in washing buffer) was then added and incubated for 60 minutes at room temperature. Excess antibody was removed by extensive washing with washing buffer and HRP activity was measured after addition of Quantablu fluorogenic peroxidase substrate (100 µL; #15169, PIERCE) and incubation for 20 minutes at room temperature. IC_{50} values were calculated from the plot of percentage of inhibition of FB activity versus inhibitor concentration using non-linear regression analysis software (XLfit, version 4.0; ID Business Solution Ltd., Guildford, Surrey, UK). Mouse FB activity was measured in an analogous way using a CVF-mouseBb complex (produced using human FD) and human C3 as substrate. Final assay concentration of CVF-mouseBb was 2 nM.

Human factor B inhibition was also tested using a competition binding assay with a Cy5 fluorescently labeled small-molecule inhibitor as probe. FB was labeled with biotin by incubating the protein at a ratio of 1:2 with EZ-Link™ Sulfo-NHS-LC-LC-Biotin (Thermo Scientific #21338) for 1 h on ice. The reaction was stopped by addition of 1 M Tris, pH 7.5, and the biotinylated FB was purified by two passages over a 2 mL Zeba™ desalt spin column (PIERCE #89890). For the assay, biotinylated FB (10 nM) was pre-incubated in the absence or presence of varying concentrations of test compound for 1 h at room temperature in assay buffer. After addition of Cy5 labeled probe (75 nM) and europium-chelate labeled streptavidin (Perkin Elmer #AD0060; 5 nM) time-resolved fluorescence energy transfer (TR-FRET) was measured using 337 nm as excitation and 665 nm as emission wavelengths (70 µs time-gated). IC_{50} values measured with the TR-FRET assay were very similar to those measured with the C3 proteolysis assay as determined by parallel measurements using multiple different inhibitor compounds.

AP complement deposition assay in serum. Serum from healthy donors was obtained under informed consent through the Novartis Basel tissue donor program and approved by the local ethics committee (EKNZ). For activation, zymosan A was suspended at 1% (w/v) in Tris-buffered saline (pH 7.6) and heated to 100 °C for 10 minutes using a water bath. The suspension was centrifuged at 4000 rpm for 30 minutes and the pellet containing the zymosan A was re-suspended in TBS buffer at 50 mg/mL. Black MaxiSorb™ plates were coated with activated zymosan A diluted to 1 mg/mL in carbonate buffer (pH 9.5, Sigma) and incubated for 2 hours at room temperature. Compounds were serially diluted in DMSO in polypropylene V-bottom plates. Normal human serum (Quidel) was diluted to 50% (v/v) with 2x gelatin buffer (0.15 mM $CaCl_2$, 141 mM NaCl, 4.5 mM $MgCl_2$, 4.2 mM HEPES, 0.1% gelatin at pH 7.4) containing 20 mM EGTA or 20 mM EDTA and added to the compound containing plate. After 30 minutes incubation at room temperature, 25 µL of the

mixture were transferred to the washed zymosan A coated plate to allow activation of the alternative pathway. The reaction plate was incubated at 37 °C for 15 minutes. The reaction was terminated by aspirating the supernatant, addition of blocking buffer (Thermo #37539) for 10 minutes and washing, and MAC formation was detected with a mouse anti-human C9 neoepitope monoclonal antibody. The antibody was added to the plate at 0.2 µg/mL in 25 mM Tris, 0.15 M NaCl, 0.05% (v/v) Tween-20, pH 7.5 (TBS-T buffer). After washing, a horseradish peroxidase (HRP)-conjugated goat anti-rat IgG (1/1000 dilution in TBS-T) antibody, cross-reacting with murine antibodies, was added to each well for 45 minutes at room temperature and, after washing, the reaction was developed using 25 µL Quanta blue for 20 minutes at room temperature. The plate was read at 325 nm excitation and 420 nm emission wavelengths in a TECAN Safire2 fluorimeter. The baseline (EDTA-treated serum, maximum inhibition control) and the maximum signal (EGTA-treated serum in the absence of compound) were used to generate percent inhibition values for each of the wells. Graphpad Prism was used to calculate the average ± standard deviation for the duplicate analysis. Inhibition was calculated as:
% inhibition = [((Maximum avg - Baseline avg)-(Test well avg – Baseline avg)) x 100]/ (Maximum avg – Baseline avg).
The IC_{50} value was calculated using GraphPad Prism software.
AP complement deposition in mouse serum (collected from internal animals) was measured in an analogous way except that the activation of the AP in zymosan A coated plates was allowed to proceed for 40 minutes, and C3b deposition was detected instead of MAC formation. For the latter, a rat anti-mouse C3b/iC3b/C3c monoclonal antibody (clone 2/11, Cell Sciences or HM1065, Hycult biotech) was used at a concentration of 2 µg/mL in TBS-T.
The rat AP deposition assay was performed as the human assay, except that mouse anti-rat C5b-9 clone 2A1 (HycultBiotech) was used as detection reagent (diluted 1/100), followed by HPR-conjugated goat anti-mouse IgG (diluted 1/1000).

Classical and lectin pathway assay in human serum. LNP023 was tested in the commercial Wieslab assay for classical and lectin pathway inhibition according to the manufacturer's instructions (Eurodiagnostica, COMPL MP 320 (lectin pathway) and CP 310 (classical pathway)). In brief, a serial dilution of LNP023 was added to serum (pooled from 3 healthy donors) that was diluted 1/100 with buffer, and pre-incubated for 15 minutes at room temperature. LNP023 was used up to a final concentration of 100 µM. The mixture was transferred to pre-coated plates and incubated for 60 minutes at 37 °C. After washing 3 times, conjugated antibody was added and incubated for 30 minutes at room temperature, followed by another round of washing. Substrate was added for 30 minutes at room temperature and optical density at 405 nm was measured using a SpectraMax M5^e ELISA reader (Molecular Devices). Anti-C5 was used as positive control.

Membrane attack complex (MAC) formation assay in 50% human whole blood. Whole blood from individual donors was collected under the Novartis employee blood donor program with informed consent from all participants. Blood was anticoagulated with the thrombin-specific anticoagulant lepirudin at 50 µg/mL (Refludan®, Bayer), then diluted by addition of an equal volume of GVB buffer (Boston BioProducts) containing 2 mM $MgCl_2$ and 10 mM EGTA (final concentration) to inhibit classical and lectin pathway activation. Compound serial dilutions were prepared in DMSO, and aliquots of the 50% blood mixture were pre-incubated in the absence or presence of compound for 15 minutes (final DMSO concentration in all test wells was 0.9% (v/v)) in a 384-well plate. A suspension of activated zymosan A (5 µL of a 10 mg/mL suspension, final concentration of 1 mg/mL, prepared as described above) was added to each well of the reaction plate to initiate AP activation, which was allowed to proceed for 50 minutes at 37 °C. Complement activation was stopped by addition of an equal volume of 0.05 M EDTA in GVB buffer and MAC generation was detected by ELISA. For this, the reaction supernatants were transferred to a 384-well ELISA plate (Nunc Maxisorp). The plate was washed with TBS-T and an alkaline phosphatase-conjugated mouse anti-human C9 neo-epitope monoclonal antibody solution was added to each well at 0.25 µg/mL. After washing, 4-MUP substrate (Fisher Scientific Inc., at 0.18 mg/mL in 0.1 M Tris–HCl buffer at pH 9.0, containing 2 mM $MgCl_2$) was added and the plate was incubated at room temperature for 30 minutes, protected from light. The reaction was stopped by addition of an equal volume of a 0.2 M EDTA solution. Plates were read at 355 nm excitation and 460 nm emission wavelengths in a TECAN Safire2 fluorimeter. Percentage of inhibition of complement deposition by the compound was calculated as above. IC_{50} values were calculated using XLFit software (formula 205 for four parameter logistic or sigmoidal dose response model).

SPR binding affinity assay. SPR experiments were performed at 20 °C using a ProteOn XPR36 instrument (Bio-Rad Laboratories). PBS (pH 7.4) supplemented with 0.005% (v/v) Tween 20 was used as running buffer. Human FB was immobilized covalently to a GLH sensor chip (Bio-Rad Laboratories) at a flow rate of 30 µL/minute using an amine coupling protocol. Reagents for the immobilization were purchased from Bio-Rad Laboratories (ProteOn Amine Coupling Kit, #1762410). The sensor chip surface was activated by a 5 minutes injection of a 1:1 (v/v) mixture of a 10 mM N-hydroxysulfosuccinimide (Sulfo-NHS) solution and a 40 mM N-(3-dimethylaminopropyl)-N-ethylcarbodiimide (EDAC) hydrochloride salt solution in water. The protein, diluted to 0.05 mg/mL in 20 mM 2-(N-morpholino) ethanesulfonic acid buffer (pH 6.0), was then injected for 5 minutes and remaining reactive groups subsequently deactivated by injecting a 1 M ethanolamine hydrochloride solution in aqueous NaOH (pH 8.5) for 5 minutes. The immobilization level of the protein was 19,500 response units (RU). To determine kinetic parameters of the binding interaction, two independent experiments were run using a Standard Kinetics

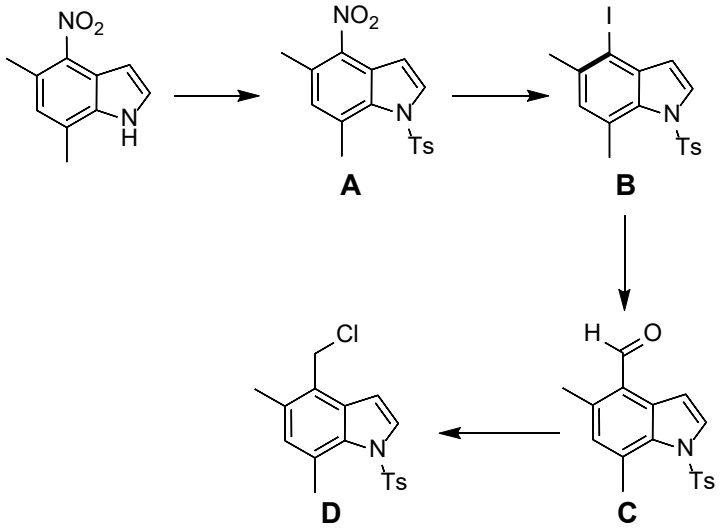
method: two-fold or three-fold serial dilutions of LNP023 were prepared ranging from 1.9 to 150 nM. Each solution was injected for 120 seconds at a flow rate of 50 µL/minute with a subsequent dissociation time of 1800 seconds. Data were fitted with ProteOn Manager software version 3.1.0.6 using a Langmuir single-site binding model.

Protein crystallography. The published crystal form of uninhibited human FB (PDB 1DLE) was found to be unsuitable for ligand soaking experiments since the active site is blocked by crystal contacts. Therefore, co-crystallization screening experiments were conducted with all compounds by using the catalytic domain (Asp470-Leu764) of human FB at a concentration of 25 mg/mL in 20 mM Tris, pH 7.5, containing 100 mM sodium chloride and 100 µM $ZnCl_2$. All compound stock solutions were at 100 mM concentration in 100% (v/v) DMSO. Crystals were grown at 20 °C using a sitting drop vapor diffusion format. Crystallization screening was carried out using an Oryx8 robot (Douglas Instruments Ltd., UK). Co-crystallization experiments for individual compounds provided suitable crystals after one week under the following conditions: for compound **1** (at 2 mM concentration), crystals grew in 2.0 M ammonium sulfate, 0.1 M sodium acetate (pH 4.6); for compound **2** (at 3 mM concentration), crystals grew in 2.5 M ammonium sulfate, 0.1 mM sodium acetate (pH 4.6); and for compound **3** (at 5 mM concentration) crystals appeared in 24% (w/v) PEG3350, 0.2 M ammonium acetate, 0.1 M Bis-Tris (pH 5.5). All co-crystallization experiments contained in addition calcium, magnesium, manganese and zinc ions at 20 µM concentrations (source: "Silver Bullet" screen condition #41, Hampton Research, USA). All crystals were cryo-cooled in liquid nitrogen without the addition of cryo-protectant. All data were collected at the PXII beamline of the SLS (PSI, Villigen, Switzerland). Data collection and refinement statistics are shown in Supplementary Table 2. Crystal structures were solved by molecular replacement using the published structure of the catalytic domain (PDB 1DLE). Structures were built and refined using Coot and Refmac.

Chemical synthesis. *General information*. Unless noted otherwise, all reagents were purchased from commercial sources and used as received. ^{1}H, ^{13}C and ^{19}F NMR were measured on various Bruker Avance spectrometers at room temperature. If not indicated otherwise, data are reported as follows: chemical shift (p.p.m., δ units) from an internal standard, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad), coupling constant (Hz), and integration. High-resolution mass analyses were performed on a Q Exactive Plus mass spectrometer (Thermo Scientific), coupled to an Ultimate 3000 UHPLC, using electrospray ionization after separation by liquid chromatography; the elemental composition was derived from the averaged mass spectra acquired at the high resolution of about 30'000 and high mass accuracy <1 ppm was obtained by using a lock mass. Low-resolution mass analyses (LRMS) were performed on

a Waters Acquity UPLC by electrospray ionization (ESI) and either negative ion or positive ion detection. Optical rotations were measured on a Perkin-Elmer 241 polarimeter.
*Characterization of compound **1***. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 (d, J = 8.3 Hz, 1H), 7.89 (d, J = 8.3 Hz, 1H), 7.53-7.50 (m, 1H), 7.47-7.44 (m, 2H), 5.93 (s br, 2H), 3.31 (s br, 4H), 2.56 (d, J = 0.6 Hz, 3H); LRMS (ESI): m/z = 304.1 $[M+H]^+$.

*Synthesis scheme for intermediate **D** (key intermediate for synthesis of compound **2**):*



*Synthesis of Intermediate **D**.*
A *5, 7-dimethyl-4-nitro-1-tosyl-1H-indole.* NaH (3.2 g, 60% in mineral oil, 79 mmol) was added portion wise to a solution of 5,7-dimethyl-4-nitro-1 *H*-indole (CAS# 1190314-35-2, 10 g, 52.6 mmol) in DMF (200 mL) at 0 °C, and the mixture was stirred at room temperature for 0.5 h. The mixture was cooled to 0 °C and TsCl (15.0 g, 79 mmol) was added followed by 22 h stirring at room temperature. The reaction was quenched with half saturated aq. $KHSO_4$, diluted with H_2O and then stirred at room temperature for 1 h. The resulting solid was collected by filtration, washed with H_2O, MeOH, and heptane and dried to give 5, 7-dimethyl-4-nitro-1-tosyl-1H-indole (17 g, 94%). MS (ESI+) m/z 345.1 (M+H).
B *4-iodo-5, 7-dimethyl-1-tosyl-1H-indole.* Zinc (16.1 g, 247 mmol) was added to a solution of 5, 7-dimethyl-4-nitro-1-tosyl-1 *H*-indole **A** (17 g, 49.4 mmol) in MeOH (50 mL)/EtOAc (300 mL). The suspension was cooled to 0 °C and AcOH (30 mL) was added dropwise over 30 minutes. The resulting mixture was stirred for 0.5 h at 0 °C and then for 18.5 h at room temperature. The reaction mixture was subsequently poured into a mixture of Celite® and 5% aq. $NaHCO_3$ in EtOAc, and was vigorously stirred for 0.5 h. The mixture was then filtered through a pad of Celite®. The filtrate layers were separated and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with 5% aq. $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, and filtered. Concentration of the filtrate gave 5, 7-dimethyl-1-tosyl-1H-indol-4-amine (15.5 g), which was used without further

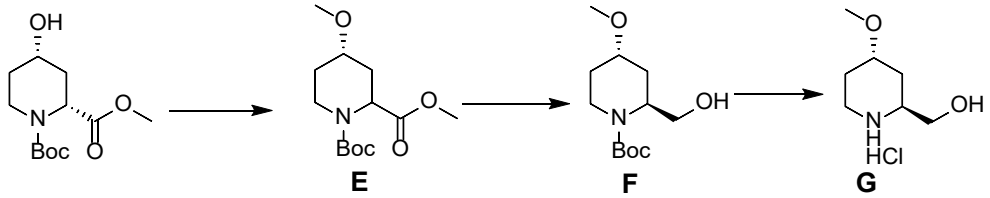
purification in the subsequent step. MS (ESI+) m/z 315.1 (M+H). Concentrated aq. HCl (4.3 mL, 49.0 mmol) was added to a suspension of 5,7-dimethyl-1 -tosyl-1 *H*-indol-4-amine (7.70 g, 24.5 mmol) in H_2O (80 mL)/EtOAc (150 mL) at 0 °C. Then a solution of $NaNO_2$ (2.0 g, 29.4 mmol) in H_2O (20 mL) was added dropwise over 15 minutes while keeping the temperature below 5 °C. Once the addition was complete, the mixture was stirred at 0 °C for 1 h. A solution of KI (12.2 g, 73.5 mmol) in H_2O (20 mL) was added dropwise to the mixture over 15 minutes and the mixture stirred at 0 °C for an additional 1 h. The reaction was quenched with half saturated $Na_2S_2O_3$, and the whole mixture was stirred at room temperature for 16 h. The mixture was diluted with EtOAc and the resulting layers were separated. The organic layer was successively washed with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by silica gel flash column chromatography [heptane/ (30% EtOAc in CH_2Cl_2) = 91/9 to 85/15)]. The resulting residue was triturated with Et_2O and the solid collected by filtration to give 4-iodo-5,7-dimethyl-1-tosyl-1H-indole (4.8 g, 46%) . ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.92 (d, J=3.80 Hz, 1 H), 7.61 (d, J=8.60 Hz, 2H), 7.40 (dd, J=0.50, 8.60 Hz, 2H), 7.04 (s, 1 H), 6.72 (d, J=3.79 Hz, 1 H), 2.41 (s, 3H), 2.37 (s, 3H), 2.34 (s, 3H).

C *5, 7-dimethyl-1-tosyl-1H-indole-4-carbaldehyde. Sec*-butyl lithium in cyclohexane (1.4 M, 7.26 mL, 10.16 mmol) was added to a solution of 4-iodo-5,7-dimethyl-1 -tosyl-1 *H*-indole **B** (1.8 g, 4.23 mmol) and DMF (0.655 mL, 8.46 mmol) in ethyl ether (200 mL) at -78 °C. After stirring for 1 h, additional *sec*-butyl lithium in cyclohexane (1.4 M, 3.63 mL, 5.08 mmol) was added. After stirring for 15 minutes, the reaction was quenched with MeOH and sat. $KHSO_4$ aqueous solution, and diluted with EtOAc and brine. The layers were separated and the aqueous layer was extracted with EtOAc. The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by silica gel flash column chromatography [(10% CH_2Cl_2/ heptane)/ (20% EtOAc/ CH_2Cl_2) = 100/0 to 50/50] to afford 5, 7-dimethyl-1-tosyl-1H-indole-4-carbaldehyde (1.0 g, 72%). MS (ESI+) m/z 328.2 (M+H).

D *4-(chloromethyl)-5, 7-dimethyl-1-tosyl-1H-indole.* $NaBH_4$ (1 g, 26.4 mmol) was added to a solution of 5, 7-dimethyl-1-tosyl-1 H-indole-4-carbaldehyde **C** (3 g, 9.16 mmol) in THF (50 mL)/MeOH (50 mL) at room temperature. The mixture was stirred at room temperature for 1.5 h and quenched with half saturated aq. $KHSO_4$. The mixture was then extracted with EtOAc/trifluoroethanol (TFE) (9/1). The organic layer was washed successively with H_2O and brine, dried over Na_2SO_4, and concentrated to furnish (5,7-dimethyl-1-tosyl-1H-indol-4-yl) methanol which was used without further purification in the subsequent step. MS (ESI-) m/z 328.2 (M-H), (ESI+) m/z 312.3 (M-OH). *N*-(chloromethylene)-*N*-methylmethanaminium chloride (CAS: 3724-43-4, 2 g, 15.62 mmol) was added to a solution of (5, 7-dimethyl-1 -tosyl-1 *H*-indol-4-yl) methanol (3 g, 9.11 mmol) in CH_2Cl_2 (80 mL) at room temperature. The mixture was stirred at room temperature for 0.75 h and then cooled to 0 °C. The reaction was quenched with 5% aq. $NaHCO_3$ at 0 °C and extracted with CH_2Cl_2. The organic layer was washed successively

with 0.2 M aq. LiCl and brine, dried over Na_2SO_4, and then concentrated. The resulting residue was triturated with Et_2O, and the resulting solid was collected by filtration to afford 4-(chloromethyl)-5, 7-dimethyl-1-tosyl-1H-indole (3.0 g, 94%). MS (ESI+) m/z 312.4 $(M-Cl)^+$.

*Synthesis scheme of intermediate **G** (key intermediate for synthesis of compound **2**):*



*Synthesis of intermediate **G**.*

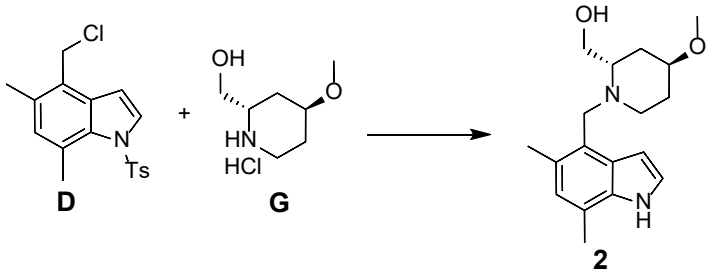
E *1-(tert-butyl) 2-methyl (4S)-4-methoxypiperidine-1, 2-dicarboxylate.* NaH, 60% in oil (135 mg, 3.37 mmol) was added to a solution of (*2R, 4S*)-1-*tert*-butyl 2-methyl 4-hydroxypiperidine-1,2-dicarboxylate (CAS# 321744-26-7, 500 mg, 1.928 mmol) in DMF (12 mL) at 0 °C. After 5 minutes, MeI (0.181 mL, 2.89 mmol) was added. The reaction mixture was stirred at 0 °C for 1.5 h and further at room temperature for 20 minutes. The reaction mixture was cooled to 0 °C, and additional NaH (80 mg) and MeI (100 µL) was added. After stirring at 0 °C for 5 minutes the reaction mixture was allowed to stir at room temperature for 20 minutes, then re-cooled to 0 °C, and quenched with saturated aq. NH_4Cl. The mixture was diluted with ether, the layers separated, and the aqueous layer was extracted again with ether. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The resulting residue was purified by silica gel flash column chromatography (heptane/EtOAc=5/1 to 5/2) to afford 1-(tert-butyl) 2-methyl (4S)-4-methoxypiperidine-1,2-dicarboxylate (473 mg, 90%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.15 – 4.56 (m, 1H), 4.25 – 3.80 (m, 1H), 3.80–3.70 (m, 3H), 3.43–3.15 (m, 4H), 2.72–2.44 (m, 1H), 2.12–1.72 (m, 2H), 1.67–1.54 (m, 1H), 1.48 (s, 9H), 1.42–1.25 (m, 1H).

F *tert-butyl (2S, 4S)-2-(hydroxymethyl)-4-methoxypiperidine-1-carboxylate.* 1 M $LiAlH_4$ in THF (2.149 mL, 2.149 mmol) was added dropwise to a solution of 1-(*tert*-butyl) 2-methyl (4S)-4-methoxypiperidine-1,2-dicarboxylate **E** (470 mg, 1.720 mmol) at -10 °C. After 15 minutes, an additional 1 mL of 1 M $LiAlH_4$ THF solution was added, followed by 10 minutes of stirring. The reaction mixture was quenched with ethyl acetate, followed by 1N aq. HCl. The mixture was stirred for 15 minutes and then filtered through Celite®. The eluent was washed with saturated aq. $NaHCO_3$, dried over MgSO4, filtered and concentrated. The resulting residue was purified by silica gel flash column chromatography (EtOAc/heptane=65% to 85%) to afford tert-butyl (2S,4S)-2-(hydroxymethyl)-4-methoxypiperidine-1-carboxylate (127 mg, 30%). 1H NMR (400 MHz, Chloroform-d) δ 5.23 (s, 1H), 4.38 (dtd, J = 8.4, 5.7, 2.1 Hz, 1H), 4.10 – 3.77 (m, 1H), 3.67 (dd, J = 11.1,

8.9 Hz, 1H), 3.56 (dd, J = 11.0, 5.6 Hz, 1H), 3.40 – 3.22 (m, 4H), 3.01 – 2.68 (m, 1H), 2.10 – 1.86 (m, 2H), 1.40 (s, 9H), 1.36 – 1.12 (m, 2H).

G *((2S, 4S)-4-methoxypiperidin-2-yl) methanol hydrochloride.* 4 N HCl in dioxane (3 mL, 12.00 mmol) was added to a solution of *tert*-butyl *(2S, 4S)*-2-(hydroxymethyl)-4-methoxypiperidine-1-carboxylate **F** (122 mg, 0.497 mmol) in dioxane (0.5 mL). The reaction was stirred for 1 h, and then concentrated to afford ((2S, 4S)-4-methoxypiperidin-2-yl) methanol hydrochloride (103 mg, quant.). 1H NMR (400 MHz, DMSO-d_6) δ 3.76 – 3.65 (m, 1H), 3.62 (q, J = 3.2 Hz, 1H), 3.53 – 3.45 (m, 2H), 3.25 (s, 3H), 3.18 (d, J = 15.2 Hz, 1H), 3.09 – 3.00 (m, 1H), 2.93 (q, J = 12.2 Hz, 1H), 1.91 (d, J = 14.6 Hz, 2H), 1.78 (d, J = 12.2 Hz, 1H), 1.69 – 1.57 (m, 1H).

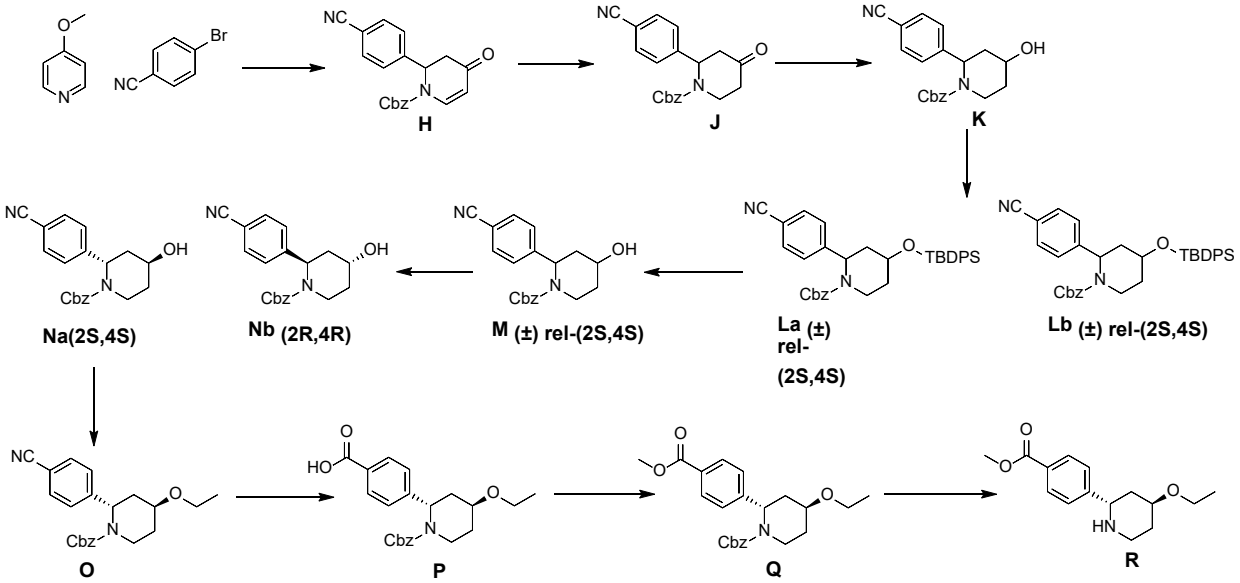
*Synthesis scheme for compound **2:***



*Synthesis of compound **2**.*

(*(2S, 4S)*-4-methoxypiperidin-2-yl) methanol hydrochloride **G** (39.4 mg, 0.216 mmol) was added to a suspension of 4-(chloromethyl)-5, 7-dimethyl-1-tosyl-1*H*-indole **D** (50 mg, 0.144 mmol) in EtOH (2 mL) followed by DIPEA (0.151 mL, 0.862 mmol). The reaction was stirred and heated under microwave irradiation at 80 °C for 45 minutes, then at 120 °C for 1 h. The reaction mixture was cooled to room temperature and solid potassium hydroxide (80 mg, 1.437 mmol) and 1 mL of ammonium hydroxide were added. The reaction was heated to 100 °C for 2 h under microwave irradiation. The reaction mixture was cooled to room temperature, diluted with brine, and extracted with dichloromethane three times. Combined organic layers were concentrated. The resulting residue was purified by HPLC (acetonitrile with 0.1% TFA/water=10% to 100%). The collected fractions were concentrated. The resulting TFA salt was diluted with dichloromethane and neutralized with sat. aq. $NaHCO_3$. The organic layer was concentrated to compound **2** (13 mg, two steps 30%). ^{1}H NMR (400 MHz, DMSO-d_6) d ppm 10.80 (br. s., 1 H), 7.21 (t, *J*=2.7 Hz, 1 H), 6.67 (s, 1 H), 6.44 - 6.57 (m, 1 H), 4.34 (t, *J*=5.2 Hz, 1 H), 4.14 (d, *J*=12.6 Hz, 1 H), 3.68 (ddd, *J*=10.5, 5.2, 5.1 Hz, 1 H), 3.55 (d, *J*=12.4 Hz, 1 H), 3.48 (ddd, *J*=11.1, 5.9, 5.7 Hz, 1 H), 3.35 - 3.44 (m, 1 H), 3.20 (s, 3 H), 2.21 - 2.47 (m, 9 H), 1.66 (t, *J*=5.2 Hz, 2 H), 1.32 - 1.52 (m, 2 H); HRMS calculated for $C_{18}H_{27}N_2O_2$ (M+H)+ 303.2067, found 303.2075.

*Synthesis scheme for intermediate **O** (key intermediate for synthesis of LNP023):*



*Synthesis of intermediate **O**.*

H *(±)-benzyl 2-(4-cyanophenyl)-4-oxo-3, 4-dihydropyridine-1(2H)-carboxylate.* Isopropylmagnesium chloride lithium chloride complex solution (1.3 M in THF, 70 mL, 91 mmol) was added dropwise over 0.25 h to a solution of 4-bromobenzonitrile (17 g, 93 mmol) in THF (50 mL) at room temperature. The mixture was stirred at room temperature for 2 h. The mixture was then diluted with THF (300 mL) and cooled to -5 °C. 4-methoxypyridine (CAS# 620-08-6, 8.37 mL, 82 mmol) was added to the mixture, followed by Cbz-Cl (12 mL, 84 mmol) while maintaining the internal temperature below 0 °C. The mixture was stirred at 0 °C for 1.5 h, and then stirred at room temperature for another 16 h. The reaction was quenched with 5 M aq. HCl and the mixture stirred at room temperature for 0.5 h followed by dilution with EtOAc. The mixture was washed twice with H_2O, 5% aq. $NaHCO_3$ and brine, dried over Na_2SO_4, filtered through a plug of silica gel, and concentrated. The resulting residue was then triturated with Et_2O (ca. 100 mL) to afford (±)-benzyl 2-(4-cyanophenyl)-4-oxo-3, 4-dihydropyridine-1(2H)-carboxylate which was used in the subsequent step without further purification (22 g, 80%). MS (ESI+) m/z 333.3 (M+H).

J *(±)-benzyl 2-(4-cyanophenyl)-4-oxopiperidine-1-carboxylate.* A suspension of (±)-benzyl 2-(4-cyanophenyl)-4-oxo-3, 4-dihydropyridine-1 (2H)-carboxylate **H** (13 g, 39.1 mmol) and zinc (5 g, 76 mmol) in AcOH (50 mL) was stirred at 100 °C for 1 h. The reaction mixture was cooled to room temperature and filtered through a plug of Celite®, which was rinsed with Et_2O. The filtrate was further diluted with Et_2O and then washed successively with H_2O, 5% aq. $NaHCO_3$ (twice), H_2O (twice), and brine, dried over Na_2SO_4, filtered, and concentrated to furnish (±)-benzyl 2-(4-cyanophenyl)-4-oxopiperidine-1-carboxylate (13.2 g, 100%) which was used in the subsequent step without further purification. MS (ESI+) m/z 335.3 (M+H).

K *Benzyl 2-(4-cyanophenyl)-4-hydroxypiperidine-1-carboxylate.* 2N $LiBH_4$ in THF (40 mL, 80.0 mmol) was added dropwise to a solution of (±)-benzyl 2-(4-cyanophenyl)-4-oxopiperidine-1-carboxylate **J** (22 g, 66 mmol) in THF (100 mL) at room temperature. The mixture was stirred at room temperature for 0.5 h and the reaction quenched with half saturated aq. $KHSO_4$ and extracted with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated to afford benzyl 2-(4-cyanophenyl)-4-hydroxypiperidine-1-carboxylate as a diastereomeric mixture (23 g, 100%) which was used in subsequent step without further purification. MS (ESI+) m/z 337.3 (M+H).

La *(±)-rel-(2S,4S)-benzyl4-((tert-butyldiphenylsilyl)oxy)-2-(4-cyanophenyl)piperidine-1–carboxylate and* ***Lb*** *(±)-rel-(2S,4R)-benzyl 4-((tert-butyldiphenylsilyl)oxy)-2-(4-cyano-phenyl) piperidine-1 –carboxylate.* Imidazole (6 g, 88 mmol) and TBDPS-Cl (22 mL, 86 mmol) was added to a solution of benzyl 2-(4-cyanophenyl)-4-hydroxypiperidine-1-carboxylate (diastereomeric mixture) **K** (33.2 g, 69 mmol) in DMF (70 mL) at room temperature. The mixture was stirred at room temperature for 20.5 h. The reaction was quenched with MeOH and extracted with EtOAc. The organic phase was washed successively with H_2O, 5% aq. $NaHCO_3$, and brine, dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by silica gel flash column chromatography (heptane/EtOAc = 86/14) to afford in the respective elution order **La** (±)-rel-(2*S*,4*S*)-benzyl 4-((tert-butyldiphenylsilyl) oxy)-2-(4-cyano-phenyl) piperidine-1-carboxylate (desired racemic mixture, 15 g, 37.8%) and **Lb** (±)-rel-(2*S*,4*R*)-benzyl 4-((tert-butyldiphenylsilyl)oxy)-2-(4-cyano-phenyl)piperidine-1 –carboxylate (12 g, 30.3%)

La (±)-rel-(2S,4S)-Benzyl 4-((tert-butyldiphenylsilyl) oxy)-2-(4-cyanophenyl) piperidine-1-carboxylate: 1 H NMR (400 MHz, CD_3CN) δ 7.62 - 7.67 (m, 2H), 7.57 - 7.62 (m, 2H), 7.27 - 7.53 (m, 13H), 6.79 -6.83 (m, 2H), 5.43 (br. d, J=4.50 Hz, 1 H), 5.06 - 5.15 (m, 2H), 4.04 - 4.12 (m, 1 H), 3.54 - 3.63 (m, 1 H), 2.60 (dt, J=3.03, 13.64 Hz, 1 H), 2.23 - 2.30 (m, 1 H), 1.79 - 1.89 (m, 2H), 1.59 (ddt, J=5.05, 10.48, 12.82 Hz, 1 H), 1 .01 (s, 9H).

Lb (±)-rel-(2*S*,4*R*)-Benzyl 4-((tert-butyldiphenylsilyl) oxy)-2-(4-cyanophenyl) piperidine-1-carboxylate: 1H NMR (400 MHz, CD_3CN) δ 7.60 - 7.64 (m, 2H), 7.22 - 7.47 (m, 17H), 5.37 (br. d, J=6.60 Hz, 1 H), 5.02 - 5.12 (m, 2H), 4.16 - 4.21 (m, 1 H), 3.99 - 4.06 (m, 1 H), 3.49 (dt, J=3.03, 13.14 Hz, 1 H), 2.34 - 2.41 (m, 1 H), 2.01 - 2.08 (m, 1 H), 1.47 - 1 .56 (m, 1 H), 1 .35 - 1 .41 (m, 1 H), 0.73 (s, 9H).

M *(±)-rel-(2S, 4S)-benzyl 2-(4-cyanophenyl)-4-hydroxypiperidine-1-carboxylate.* (±)-rel-(2*S*, 4*S*)-benzyl 4-((tert-butyldiphenylsilyl) oxy)-2-(4-cyanophenyl) piperidine-1-carboxylate **La** (3.5 g, 6.09 mmol) was added to a solution of TBAF in THF (1 M, 20 mL, 20 mmol). The mixture was stirred at room temperature for 1.5 h and then diluted with Et_2O. The mixture was washed successively with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated to give (±)-rel-(2S,4S)-benzyl 2-(4-cyanophenyl)-4-hydroxypiperidine-1-carboxylate (2.05 g, 100%). 1H NMR (400 MHz, CD_3CN) δ 7.64 - 7.73 (m, 2H), 7.37 - 7.42 (m, 2H), 7.28 - 7.36 (m, 5H), 5.57 (br. d, J=5.00 Hz, 1 H), 5.09 -

5.18 (m, 2H), 4.12 - 4.19 (m, 1 H), 3.45 - 3.55 (m, 1 H), 2.89 (d, J=4.52 Hz, 1 H), 2.82 (dt, J=3.06, 13.51 Hz, 1 H), 2.45 - 2.53 (m, 1 H), 1 .71 - 1 .84 (m, 2H), 1 .31 - 1 .44 (m, 1 H).

Na *(-)-(2S, 4S)-benzyl 2-(4-cyanophenyl)-4-hydroxypiperidine-1-carboxylate and* ***Nb*** *(+)-(2R, 4R)-benzyl 2-(4-cyanophenyl)-4-hydroxypiperidine-1-carboxylate.* Resolution of the enantiomers of (±)-rel-(2*S*,4*S*)-benzyl 2-(4-cyanophenyl)-4-hydroxypiperidine-1-carboxylate **M** (2.7 g, 8.03 mmol) was achieved by chiral SFC using a CHIRALPAK® AD-H column with 25% (MeOH with 5 mM NH_4OH) in CO_2 to give **Nb** (+)-(2*R*,4*R*)-benzyl 2-(4-cyanophenyl)-4-hydroxypiperidine-1-carboxylate (peak-1 , tr = 2.8 minutes) (800 mg, 29.6%) and **Na** (-)-(2*S*, 4*S*)-benzyl 2-(4-cyanophenyl)-4-hydroxypiperidine-1 -carboxylate (peak-2, tr = 4.5 minutes) (900 mg, 30.3%).

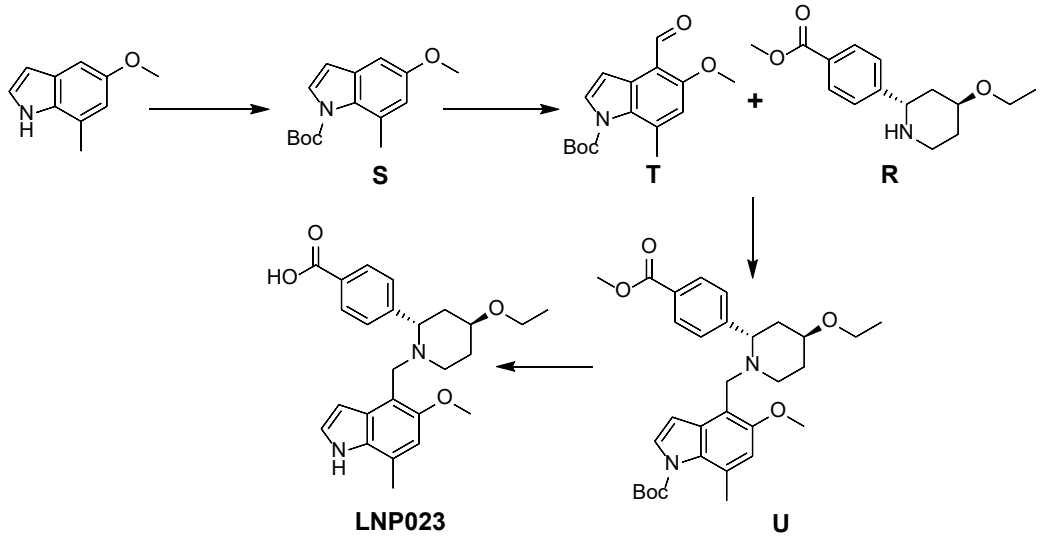
O *(2S, 4S)-benzyl 2-(4-cyanophenyl)-4-ethoxypiperidine-1-carboxylate.* EtI (1 mL, 12.37 mmol) was added to a solution of (-)-(2*S*, 4*S*)-benzyl 2-(4-cyanophenyl)-4-hydroxypiperidine-1-carboxylate **Na** (2 g, 5.95 mmol) in DMF (20 mL) at 0 °C, followed by NaH (60% in oil, 400 mg, 10 mmol). The mixture was stirred at 15 °C for 1.5 h. The reaction was quenched with MeOH and stirred for 0.25 h. The mixture was diluted with half saturated aq. $KHSO_4$ and extracted with EtOAc. The organic phase was washed successively with H_2O, 0.5M aq. LiCl, and brine, and then dried over Na_2SO_4, filtered, and concentrated to furnish (2S,4S)-benzyl 2-(4-cyanophenyl)-4-ethoxypiperidine-1-carboxylate (2.17 g) which was used in the next step without further purification. MS (ESI+) m/z 365.3 (M+H).

P *4-((2S, 4S)-1 -((benzyloxy)carbonyl)-4-ethoxypiperidin-2-yl)benzoic acid.* A suspension of (2*S*, 4*S*)-benzyl 2-(4-cyanophenyl)-4-ethoxypiperidine-1-carboxylate **O** (2.17 g, 5.95 mmol) and $Ba(OH)_2$ hexahydrate (6 g, 21.5 mmol) in iPrOH/H_2O (15 mL/40 mL) was stirred at 100 °C for 20 h and then cooled to room temperature. The reaction mixture was acidified with half saturated aq. $KHSO_4$ and extracted with EtOAc. The organic layer was washed successively with H_2O (twice) and brine, dried over Na_2SO_4, filtered, and concentrated to furnish 4-((2S, 4S)-1-((benzyloxy)carbonyl)-4-ethoxypiperidin-2-yl)benzoic acid (2.26 g) which was used in the next step without further purification. MS (ESI+) m/z 384.3 (M+H).

Q *(2S, 4S)-benzyl 4-ethoxy-2-(4-(methoxycarbonyl) phenyl) piperidine-1-carboxylate.* $SOCl_2$ (1 mL, 13.70 mmol) was added dropwise to MeOH (10 mL) at 0 °C. To the resulting methanolic HCl solution at room temperature a solution of 4-((2*S*, 4*S*)-1-((benzyloxy) carbonyl)-4-ethoxypiperidin-2-yl) benzoic acid **P** (2.262 g, 5.9 mmol) in MeOH (10 mL) was added dropwise. The mixture was stirred at 40 °C for 2 h and then stirred at room temperature for 16 h. The reaction mixture was diluted with CH_2Cl_2 and the organic phase washed successively with 5% aq. $NaHCO_3$ (twice), H_2O, and brine, dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by silica gel flash column chromatography (heptane/EtOAc = 68/32) to afford (2S, 4S)-benzyl 4-ethoxy-2-(4-(methoxycarbonyl) phenyl) piperidine-1-carboxylate (1.8 g, 4.53 mmol, three steps, yield of 66.7%).

R *Methyl 4-((2S, 4S)-4-ethoxypiperidin-2-yl) benzoate.* A mixture of (2*S*, 4*S*)-benzyl 4-ethoxy-2-(4-(methoxycarbonyl) phenyl) piperidine-1-carboxylate **Q** (1.45 g, 3.65 mmol) and Pd/C (5%) (0.776 g, 0.365 mmol) in MeOH (20 mL) was stirred at room temperature under a H_2 atmosphere for 5 h. The H_2 gas was then replaced with N_2 and the catalyst was removed by filtration through a plug of Celite®. The filtrate was concentrated to furnish methyl 4-((2S, 4S)-4-ethoxypiperidin-2-yl) benzoate (870 mg, 91%). MS (ESI+) m/z 264.3 (M+H).

Synthesis scheme for LNP023:



Synthesis of ***LNP023****.*

S *tert-butyl 5-methoxy-7-methyl-1 H-indole-1-carboxylate.* Boc_2O (18.52 g, 85 mmol) and DMAP (10.37 g, 85 mmol) was added to a solution of 5-methoxy-7-methyl-1 H-indole (CAS: 61019-05-4, 11.40 g, 70.7 mmol) in acetonitrile (100 mL) at room temperature. The mixture was stirred for 40 minutes and then diluted with CH_2Cl_2 and saturated aq. NH_4Cl. The resulting layers were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by silica gel flash column chromatography (0 to 100% EtOAc/heptane) to provide tert-butyl 5-methoxy-7-methyl-1 H-indole-1-carboxylate (18 g, 97%). MS (ESI+) m/z 262.2 (M+H).

T *tert-butyl 4-formyl-5-methoxy-7-methyl-1 H-indole-1-carboxylate.* Oxalyl chloride (7.44 mL, 85 mmol) was added dropwise to a solution of N-methylformanilide (10.49 mL, 85 mmol) in CH_2Cl_2 (68 mL) at room temperature over 30 minutes. The mixture was stirred for 16 h at room temperature. The mixture was then added dropwise over 45 minutes to a solution of tert-butyl 5-methoxy-7-methyl-1 H-indole-1-carboxylate **S** (16.99 g, 65 mmol) in CH_2Cl_2 (70 mL) at -14 °C. The resulting mixture was stirred for 1.5 h at -14 °C, the reaction quenched with ice water, and the mixture extracted three times with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and

concentrated. The resulting residue was purified by silica gel flash column chromatography (0-25% EtOAc/heptane) to provide tert-butyl 4-formyl-5-methoxy-7-methyl-1 H-indole-1-carboxylate (12.2 g, 64.9%). MS (ESI+) m/z 290.1 (M+H).

U *tert-butyl 4-(((2S, 4S)-4-ethoxy-2-(4-(methoxycarbonyl)phenyl)piperidin-1-yl)methyl)-5-methoxy-7-methyl-1H-indole-1-carboxylate.* $NaBH(OAc)_3$ (1.5 g, 7.08 mmol) was added to a solution of tert-butyl 4-formyl-5-methoxy-7-methyl-1 H-indole-1-carboxylate **T** (1 g, 3.46 mmol) and methyl 4-((2*S*, 4*S*)-4-ethoxypiperidin-2-yl)benzoate **R** (560 g, 2.13 mmol) in DCE (20 mL). The mixture was stirred at room temperature for 20 h. The reaction mixture was diluted with EtOAc and washed successively with 5% aq. $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by basic alumina gel flash column chromatography [CH_2Cl_2/(10% MeOH in EtOAc) = 94/6] to afford tert-butyl 4-(((2S, 4S)-4-ethoxy-2-(4-(methoxycarbonyl) phenyl) piperidin-1-yl) methyl)-5-methoxy-7-methyl-1H-indole-1-carboxylate (1.2 g, 80%). MS (ESI+) m/z 537.4 (M+1).

LNP023 *4-((2S, 4S)-(4-ethoxy-1-((5-methoxy-7-methyl-1 H-indol-4-yl) methyl) piperidin-2-yl)) benzoic acid.* A mixture of methyl 4-((2*S*, 4*S*)-4-ethoxy-1-((5-methoxy-7-methyl-1 H-indol-4-yl) methyl) piperidin-2-yl) benzoate **U** (950 mg, 1.77 mmol) and 1 M LiOH in H_2O (5 mL, 5 mmol) in THF (5 mL)/MeOH (10 mL) was stirred at room temperature for 16 h and then directly concentrated. The resulting residue was purified by RP-HPLC (Waters SunFire™ Prep C18 OBD™ 5pm, 30x100 mm column; gradient: water with 0. 1% TFA / acetonitrile) to afford 4-((2S, 4S)-(4-ethoxy-1 -((5-methoxy-7-methyl-1 H-indol-4-yl) methyl) piperidin-2-yl)) benzoic acid (470 mg, 61.9%). 1H NMR (TFA salt, 400 MHz, D_2O) δ 8.12 (d, J=8.19 Hz, 2H), 7.66 (br. d, J=8.20 Hz, 2H), 7.35 (d, J=3.06 Hz, 1 H), 6.67 (s, 1 H), 6.25 (d, J=3.06 Hz, 1 H), 4.65 (dd, J=4.28, 1 1 .49 Hz, 1 H), 4.04 (d, J=13.00 Hz, 1 H), 3.87 - 3.98 (m, 2H), 3.53 - 3.69 (m, 5H), 3.38 - 3.50 (m, 1 H), 3.20 - 3.35 (m, 1 H), 2.40 (s, 3H), 2.17 - 2.33 (m, 2H), 2.08 (br. d, J=15.70 Hz, 1 H), 1.82 - 1.99 (m, 1 H), 1 .28 (t, J=7.03 Hz, 3H); HRMS calculated for C26H31 N2O3 (M+H)+ 423.2284, found 423.2263.

LPS-induced complement activation in mice. Female 6 week old C57BL/6 mice were purchased from Charles River Laboratories (Wilmington, MA, USA). Mice were housed in a pathogen-free facility on a 12 h light/dark cycle. Mice were fed with standard rodent laboratory chow and sterile water *ad libitum*. Protocols, handling and care of the mice were in accordance with the policy of the NIBR Cambridge Animal Care and Use Committee. Studies were performed with 7-week old mice weighing approximately 20 grams. Complement activity was induced by intraperitoneal injection of 50 µg of lipopolysaccharide (LPS) from Salmonella typhimurium (Sigma) dissolved in 100 µL sterile PBS per animal. In these studies, negative control animals received an intraperitoneal injection of 100 µL sterile normal saline solution, and dosing vehicle alone by oral gavage. Positive control animals received intraperitoneal LPS and dosing vehicle

alone by oral gavage. For compound administration, a suspension formulation using 0.5% (w/v) methylcellulose and 0.5% (v/v) Tween 80 in water was prepared. For dose response studies, LPS (or PBS) was administered 7.5 h prior to end of study (time = 0), and compound at various doses (or vehicle) was administered by oral gavage 3.5 h later, or 4 h prior to the end of the study. For duration of action studies, groups of mice (grouped per time point) were given one dose of compound by oral gavage at 24, 16, 12, 10, 8, or 4 h prior to the end of the study (time = 0). All groups were given LPS by intraperitoneal injection 7.5 h prior to the end of the study. Data are presented as group mean ± SEM. Groups consisted of four mice. Mean differences were compared and considered statistically significant when $p < 0.05$ measured by one-way analysis of variance, followed by correction for multiple comparisons using Dunnett's post-test, where $*p<0.05$, $**p<0.01$, or $***p<0.001$ compared to the LPS+vehicle treated positive control group. Data was analyzed by subtracting the baseline values of C3d+iC3b levels measured in the PBS plus vehicle treated groups from the respective values for each LPS treated group. Using the baseline-subtracted values, the percent inhibition was calculated as: % inhibition = (1 – (value of C3d+iC3b with compound and LPS) / average value of C3d+iC3b in LPS plus vehicle group) x 100.

Pharmacokinetic studies. Animal studies were performed in accordance with the policy of the NIBR Cambridge Animal Care and Use Committee. The pharmacokinetics of LNP023 were determined in C57BL/6 mice (n=2 per route of administration) following a single intravenous (iv) (1 mg per kg body weight) and a single peroral (po) (10 mg per kg body weight) dose, respectively. The iv formulation was prepared as a 1.0 mg/mL solution containing 10% (v/v) propylene glycol, 25% of a 20% solutol solution in phosphate buffered saline, and animals were dosed by injection into the cannula of the jugular vein. The po group received LNP023 as a suspension (0.5% (w/v) methyl cellulose and 0.5% (v/v) Tween 80). Approximately 50 µL of whole blood were collected from the tail at 5 minutes (iv dose only), 15 minutes (po dose only), 0.5, 1, 2, 4, and 7 hours post-dose and were transferred to EDTA-coated tubes. Blood was centrifuged at 3,000 rpm and the resultant plasma was transferred to a PCR 96-well plate, capped with a PCR strip cap and frozen at –20 °C until further analysis. High performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) methods were developed and used to quantify the compound concentrations in plasma. Plasma aliquots (25 µL) were subjected to protein precipitation by adding acetonitrile (150 µL) containing 50 ng/mL of an internal standard (glyburide), followed by vortexing for 10 minutes and centrifugation for 5 minutes at 4,000 rpm (2,800 g) at 4 °C. The supernatant (125 µL) from each well was transferred to a new plate and diluted by addition of 50 µL of water. These extracts were treated as unknown samples and were measured against calibration standards prepared from a pure authentic reference standard of LNP023 in positive electrospray ionization mode. HPLC-MS/MS analyses were conducted using a MAC-MOD ACE C18 column (2.1 x 30 mm,

5µm; Mac-Mod Analytical, Chadds Ford, PA, USA) on a Sciex API-4000 mass spectrometer (Sciex, Framingham, MA, USA) with short gradient elution method with two mobile phases: water with 0.1% (v/v) formic acid and acetonitrile with 0.1% (v/v) formic acid. Multiple reaction monitoring transitions were m/z 423.4 → m/z 174.1 and m/z 423.4 → m/z 144.1 for LNP023. Glyburide was monitored by measuring transitions of m/z 494.1 → m/z 169.2. Peak integration and data analyses were carried on Analyst software v 1.6.2 (Sciex, Framingham, MA, USA) and Watson LIMS (Thermo Fisher Scientific, Waltham, MA, USA). Declustering potential and collision energy were 85 V, 55 V and 40 V, 15 V for the LNP023 transitions and 71 V and 40 V for the glyburide transitions respectively. Relevant pharmacokinetic parameters, including the area under the curve (AUC), were calculated using the linear trapezoidal rule. Extrapolated initial concentration (C_0), maximal concentration (C_{max}), volume of distribution (V_{ss}), clearance (CL) and terminal half-life ($T_{1/2}$), were estimated using non-compartmental methods using Watson LIMS software (Thermo Fisher Scientific, Waltham, MA, USA).

KRN arthritis model. Animal studies were performed in accordance with the Basel Cantonal Veterinary Committee (Basel, Switzerland). Arthritis was induced by tail vein intravenous injection of 150 µL K/BxN serum. Animals were orally gavaged with vehicle (0.5% (w/v) methyl cellulose (MC), 0.5% (v/v) Tween 80) or LNP023 compound formulation (in 0.5% (w/v) MC, 0.5% (v/v) Tween 80) at 20, 60 or 180 mg/kg/day twice daily (bid) at 12 h intervals. Compound or vehicle dosing was initiated 1 hour prior to induction with K/BxN serum and continued bid until the end of the experiment on day 6. Swelling of hind paws was scored on days 1, 4, 5 and 6 on a composite scoring scale of 0-24 per mouse. Evaluation was performed on each paw by visual inspection in the metatarsal region with score 0-3, and in the ankle with score 0-3, resulting in a maximal score of 6 per paw. The scoring numbers were defined as follows: 0 = no detectable signs of inflammation; 1 = slightly swollen with redness; 2 = more extensively swollen region; 3 = ankylosis or severely swollen region. These assessments were performed by two trained individuals. The paw scores were summed up to obtain a score for each individual animal (maximum score 24 per animal). Each group was averaged and standard errors of the mean (SEM) were calculated. Front paws were snap frozen for subsequent lysate preparation for further analyses, hind paws were taken for histopathological processing, and serum was taken for ELISA and Western blotting. For histological analysis, hind paws were fixed in 10% (v/v) buffered formalin for 48 h, decalcified over 6 days in Immunocal (Decal Chemical Corp, Tallman, NY 10982-0916 USA) changed every second day, processed, and embedded in paraffin (paraplast tissue embedding medium; Leica Microsystems, Buffalo Grove, IL). Three-micrometer thick sections were stained with hematoxylin & eosin (H&E) and safranin O. Histopathological changes were blindly scored on a scale of 0 (normal) to 3 (severe changes). The following parameters were assessed: inflammatory cell infiltrates, joint damage/bone erosions and proteoglycan loss.

Passive Heymann nephritis. Animal studies were performed in accordance with the Basel Cantonal Veterinary Committee (Basel, Switzerland). Passive Heymann nephritis was induced by tail vein intravenous injection of sheep anti-rat Fx1A serum (5 mL/kg) into 7-week old male Sprague-Dawley rats (Janvier, France). Nine animals were used per treatment group and 4-5 animals received control serum. Animals were orally gavaged with vehicle (0.5% (w/v) methyl cellulose (MC), 0.5% (v/v) Tween 80) or LNP023 compound formulation (in 0.5% (w/v) MC, 0.5% (v/v) Tween 80) at 20 or 60 mg/kg/day twice daily (bid) at 12 hour intervals. Compound or vehicle dosing was initiated 1 hour prior to induction with Fx1A serum or on day 6 (a.m.) and continued bid until the end of the experiment on day 14. For collection of urine, individual rats were put into cages containing LabSand® for 2 hours on days -2, 4, 6, 8, 11, and 13 (therapeutic) or 14 (prophylactic). Urine was collected every hour and stored on ice, then both urine samples of each animal were combined and frozen. Creatinin, albumin and total protein were measured on COBAS 6000 (Roche Diagnostics, Switzerland). Blood was sampled on days 1 and 14 for pharmacokinetic analysis, and EDTA plasma was collected on days -1, 1 and 14 for analysis of complement activation fragments. At day 14 (therapeutic study) or day 15 (prophylactic study) post anti-Fx1A injection, a necropsy was performed and kidneys were collected, weighed (absolute and relative to body weight) and processed for histopathology. Hematoxylin & eosin (H&E) sections of kidneys were submitted for microscopic evaluation and graded by a pathologist on a severity scale of 1 to 5 (1 = minimal, 2 = mild, 3 = moderate, 4 = marked and 5 = severe). Anti-c3 antibody (DAKO) was used on OCT embedded frozen kidney tissue sections to measure complement deposition in the glomerulus. *P* values were calculated using one-way ANOVA followed by Dunnett's multiple comparison test with a single pooled variance (for complement activation fragments and histology). For the proteinuria measurements, repeat measure two-way ANOVA followed by Dunnett' multiple comparison test was performed using GraphPad Prism.

Inhibition of complement activity in C3G patient serum. Serum was collected from patients with C3G or healthy volunteers and all patients gave informed consent before donating samples. Further details on the individual patient sera are summarized in the table below.

C3 cleavage products were detected by immunofixation electrophoresis (IFE). In brief, 10 µL of normal human serum were mixed with 10 µL of patient serum in PBS containing 10 mM EGTA-Mg^{2+} or 10 mM EDTA (as a control) and incubated for 45 minutes at 37 °C. C3 or C3 cleavage products were resolved by electrophoresis on precast agarose Titan gels (Helena Laboratories, Beaumont, TX). After electrophoresis, anti-human C3 antibody was added and gels were stained with acid blue and de-stained. The intensity of the bands of the C3 cleavage products and full-length C3 were quantified using AlphaEaseFC software

(Cell Biosciences, Santa Clara, CA). Experiments were included only if the maximal cleavage (in the presence of EGTA) was at least 2.5-fold higher than the background cleavage (in the presence of EDTA). Shown are the results obtained using sera from 9 patients with C3G (n = 5 for C3 glomerulonephritis and n=4 for dense deposit disease). Statistical analysis was performed with GraphPad Prism using repeated measurement one-way ANOVA with the Greenhouse Geisser correction and uncorrected Fisher's LSD.

Non-sensitized sheep erythrocytes were incubated in 20% patient serum (in the presence of compounds or vehicle) at 37 °C for 30 minutes, and hemolysis was quantified by measuring the optical density (OD) in the supernatant at 415 nm. Inhibition of hemolysis was calculated as: $(1-(OD_{EGTA+cpd} - OD_{EDTA})/(OD_{EGTAveh} - OD_{EDTA}))$. Experiments were included only if the maximal hemolysis (in the presence of EGTA) was at least 2.5-fold higher than the background hemolysis (in the presence of EDTA). Shown are results using sera from 7 patients with C3G (n = 4 for C3 glomerulonephritis and n = 3 for dense deposit disease). Statistical analysis was performed withGraphPad Prism using repeated measurement one-way ANOVA with the Greenhouse Geisser correction and Dunnett's multiple comparison test.

To test whether LNP023 inhibits active C3 convertase stabilized with C3 nephritic factors (C3Nefs), a C3 convertase stabilization assay was employed. C3b was deposited on washed sheep erythrocytes (1 mL, 1 x 10^9/mL) by incubation with 45 mL of FB-partially inactivated/FH-depleted serum at 37 °C for 30 minutes. Cells were then washed three times with GVB-EGTA- Mg^{2+} buffer and re-suspended in 1 mL of the washing buffer. For each batch of cells, the amount of FB was titrated out (from 0.4 to 2 µg) with constant FD (0.3 µg) in 0.2 mL of C3b-coated sheep erythrocytes (1 x 10^9/mL). After incubation at 30 °C for 5 minutes, 0.3 mL ice-cooled GVB-EDTA buffer was added. An aliquot (50 µL) was subsequently mixed with 50 µL of rat EDTA serum (1:9 diluted in GVB-EDTA buffer) as a source of C5-C9. The degree of lysis in the supernatant was measured by optical density at 415 nm after 1 hour incubation at 37 °C. The amount of FB was adjusted to obtain 1 hemolytic site per cell (Z = 1; Z = -ln(1-hemolysis)). To perform the assay, 500 µg of patient IgG (containing C3Nefs) in 220 µL of EDTA-GVB buffer was mixed with 30 µL of C3 convertase-coated sheep erythrocytes (Z = 1; 1×10^9/mL). C3 convertase was allowed to decay at 30 °C (water bath) for 0, 7.5, 15, and 22.5 minutes, and, at each time point, 50 µL of the mixture was transferred to an empty 96-well plate. Hemolysis was induced by the addition of 50 µL of rat EDTA serum in the absence of inhibitor or containing either LNP023 or a Factor D inhibitor (final concentration of 150 nM). Rat serum diluted 1:9 in GVB-EDTA buffer served as a source of C5-C9. Hemolytic data at 15 minutes are shown in Fig. 4*C*. Percent of C3 convertase stabilized was calculated as $(Z_{15min}/Z_{0min} \times 100)$.

Details on patient sera:

Patient	Diagnosis[1]	Disease drivers		Patient serum used for[4]			Biomarker[5]	
		Acquired[2]	Genetic[3]	IFE	HA	C3CSA	C3	sC5b-9
1	DDD	C3Nef		√	√	√	0.2	0.23
2	DDD	C3Nef		√		√	< 0.16	ND[#]
3	DDD	C3Nef		√		√	0.4	0.16
4	C3GN	To be identified[*]		√			0.6	0.77
5	C3GN	To be identified[*]		√			1	0.62
6	C3GN	FHAA		√			0.4	0.48
7	C3GN	FHAA		√			0.8	1.4
8	C3GN	FHAA/FBAA			√		0.9	0.14
9	C3GN	C4Nef			√		< 0.16	1.5
10	C3GN		*CFB* p.R203Q		√		0.53	0.38
11	C3GN				√		1.7	0.2
12	DDD	C3Nef			√	√	< 0.16	0.29
13	DDD	C4Nef			√		0.2	6.24
14	DDD	C3Nef				√	1.6	0.15
15	C3GN	C3Nef				√	0.4	0.31
16	C3GN	C3Nef				√	< 0.16	3.2
17	C3GN	C3Nef/C4Nef				√	0.2	0.18
18	C3GN	C3Nef				√	< 0.16	2.6
19	C3GN	C5Nef				√	< 0.16	5.3
20	C3GN	FBAA		√			0.5	0.3
21	DDD	FBAA		√			0.6	ND

[1]DDD = Dense deposit disease; C3GN = C3 Glomerulonephritis
[2]C3Nef = C3 Nephritic factors; FHAA = Factor H autoantibodies; FBAA = Factor B autoantibodies; C4Nef = C4 Nephritic factors; C5Nef = C5 Nephritic factors.
[3]Ultra-rare/novel variants in 5 complement genes (CFH, CFI, MCP, C3, CFB); all patients have 2 copies of *CFH, CFHR3, CFHR1, CFHR4, CFHR2 and CFHR5*
[4]IFE = Immunofixation electrophoresis; HA = Sheep erythrocyte hemolytic assay; C3CSA = C3 convertase stabilizing assay
[5]Normal reference range: C3 = 0.9-1.8 g/L; soluble C5b-9 <0.3 mg/L.
[*]Serum from these patients stabilizes C3 convertase, but the autoantibody has not been identified
[#]ND = Not done

***Ex vivo* hemolysis and C3-opsonization of PNH erythrocytes.** Peripheral blood was collected from healthy volunteers (as a source for ABO-matched normal sera) and from PNH patients. For consistency, blood was serially drawn from the same three untreated patients who exhibited a large PNH cell population (>10% on erythrocytes, >50% on granulocytes) for all experiments. Blood was collected in standard EDTA and serum Vacutainer tubes (BD Pharmingen) after venipuncture according to standard procedures and following informed consent as approved by the local IRB. This study was conducted in accordance with the Declaration of Helsinki. Erythrocytes were obtained from peripheral blood after three washings in saline and were subsequently incubated with Mg^{2+}-

supplemented sera from ABO-matched healthy individuals (normal human serum; NHS) at final hematocrits of 2%. In order to reduce inter-experiment variability, pooled sera from at least three subjects all with plasma C3 levels in the normal range (0.9-1.8 g/L) were used. AP activation was achieved by acidification using HCl (1:20 of 0.1 N HCl), which resulted in a drop in the pH value to between 6.7 to 6.9. Compound was added to the tubes at different concentrations before complement activation. After 24 h incubation time at 37 °C in acidified NHS (AcNHS), hemolysis was quantified by flow cytometry after staining of the erythrocyte pellet with a FITC-conjugated anti-C3 polyclonal antibody (Ab14396; Abcam) and a PE-conjugated anti-CD59 monoclonal antibody (59-PE; Valter Occhiena). Hemolysis was calculated by comparing baseline (pre) and post-incubation (post) percentage of PNH erythrocytes. The proportion of surviving PNH erythrocytes (% PNH post/% PNH pre) was normalized, based on the determination of residual normal RBCs (dividing by % N post/% N pre). The rate of hemolysis was then calculated as the reciprocal of the percentage of survival: % lysis = 100 − (% PNH post/% N post) × (% N pre/% PNH pre) × 100.

The same anti-C3 and anti-CD59 staining also served to assess opsonization by evaluating the deposition of C3 fragments on erythrocytes exposed to AP activation in the presence of inhibitors using flow cytometry at the end of the hemolytic assay. The anti-C3 used in this study (Abcam, Ab14396) has been shown to bind both C3b and its degradation fragments iC3b and C3dg. Inhibition was calculated by determining the average percent hemolysis of PNH erythrocytes at the indicated conditions for three individual patients (2 to 14 experiments were performed per patient). Finally, the individual mean result for each patient was averaged and SEM was calculated with GraphPad Prism. Statistical analysis was repeated measurement one-way ANOVA with the Greenhouse Geisser correction and Dunnett’s multiple comparison test using GraphPad Prism.

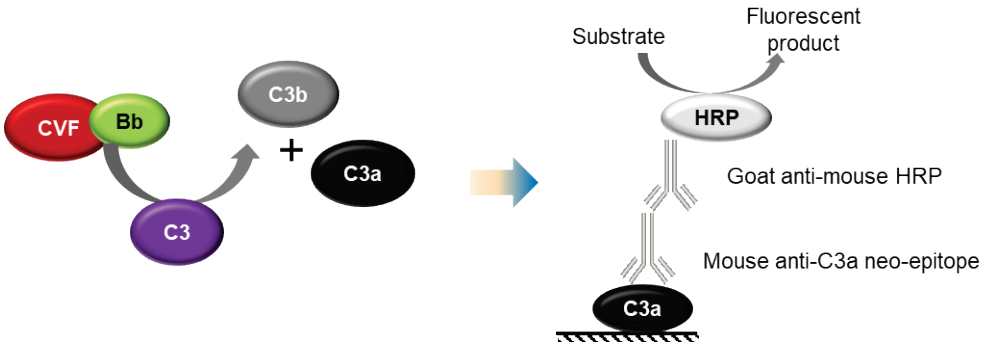
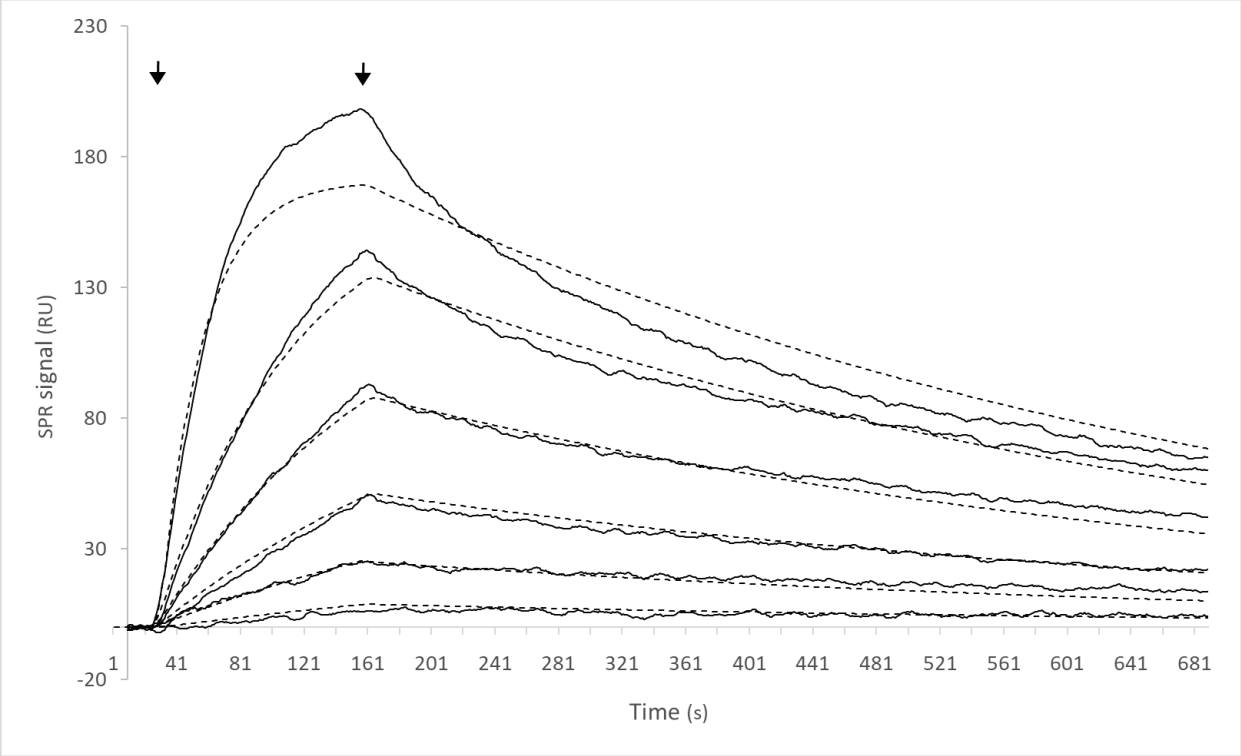


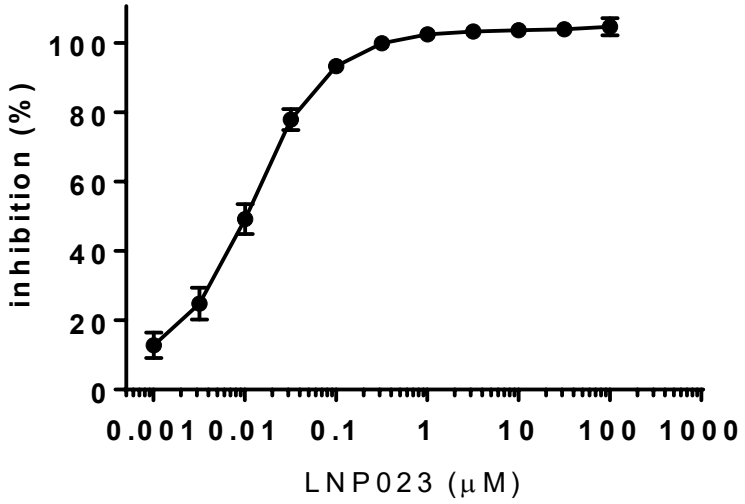
Fig. S1. Design of the FB activity assay. The assay is based on the proteolysis of complement C3 by the CVF:Bb complex and relies on the detection of *de novo* formed C3a protein using an ELISA assay format. The C3a cleavage product is adsorbed on high-capacity binding 384-well microtiter plates and quantified using a primary mouse anti-C3a neo-epitope antibody, followed by a secondary goat anti-mouse antibody linked to horseradish peroxidase (HRP).



	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_D (μM)
Experiment 1	2.23 x 10^5	1.90 x 10^{-3}	0.0085
Experiment 2	2.63 x 10^5	1.91 x 10^{-3}	0.0073

Fig. S2. Binding affinity of LNP023 to human factor B. SPR sensorgrams showing binding of increasing concentrations of LNP023 (from top to bottom: 150, 50, 25, 12.5, 5.6, and 1.9 nM) to immobilized human FB. Each concentration was injected for 120 seconds followed by a dissociation time of 1800 seconds. Beginning and end of compound injections are shown by the black arrows at the top of the graph. Shown are the graphs of one experiment (solid lines) with data fitted using a Langmuir single site binding model (dashed lines); kinetic data are from 2 independent experiments.

A



B

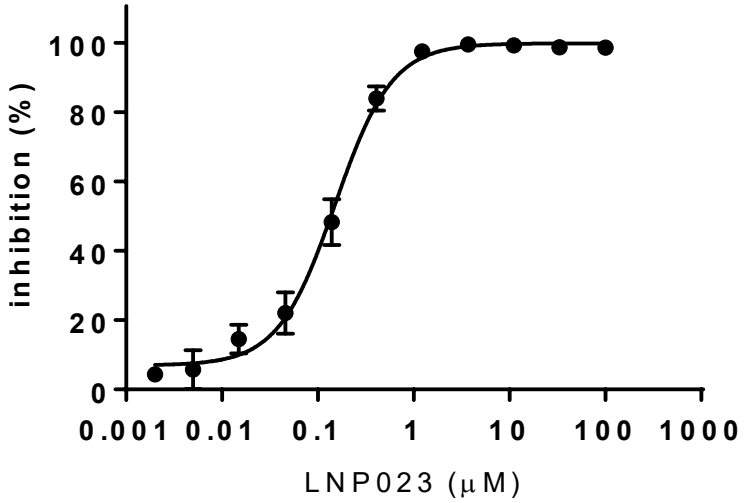


Fig. S3. *In vitro* potency of LNP023. (*A*) Determination of IC_{50} value for inhibition of human FB (n = 22) following the proteolysis of C3 by the CVF:Bb complex at different LNP023 concentrations. (*B*) Concentration-dependent inhibition of AP-mediated MAC formation in 50% normal human whole blood from individual donors. Representative curve. Data are mean ± SD of 3 replicates from 2 individual donors per compound. All IC_{50} values are summarized in Table S3.

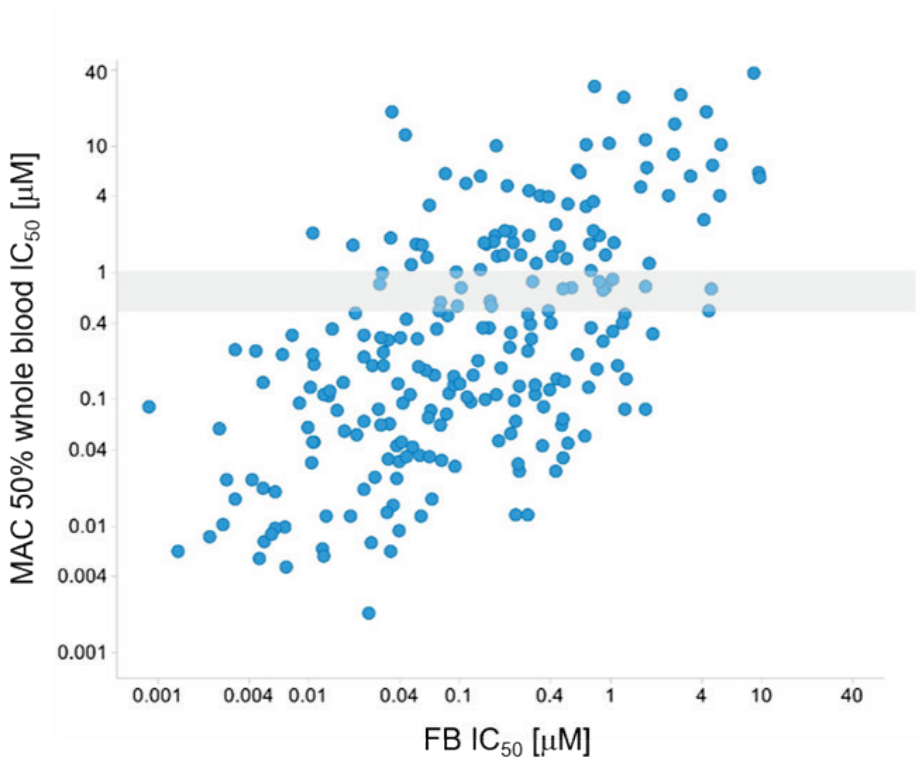


Fig. S4. Correlation between IC_{50} values for FB inhibition and those for inhibition of MAC formation. Compounds are derivatives of compound **1** and **2** generated during compound optimization. Inhibition was determined by following the proteolysis of C3 by the CVF:Bb complex (FB) and detecting AP-mediated MAC formation in 50% human blood. The range of the predicted endogenous FB concentration (50% of 1-2 µM) is indicated by the grey bar.

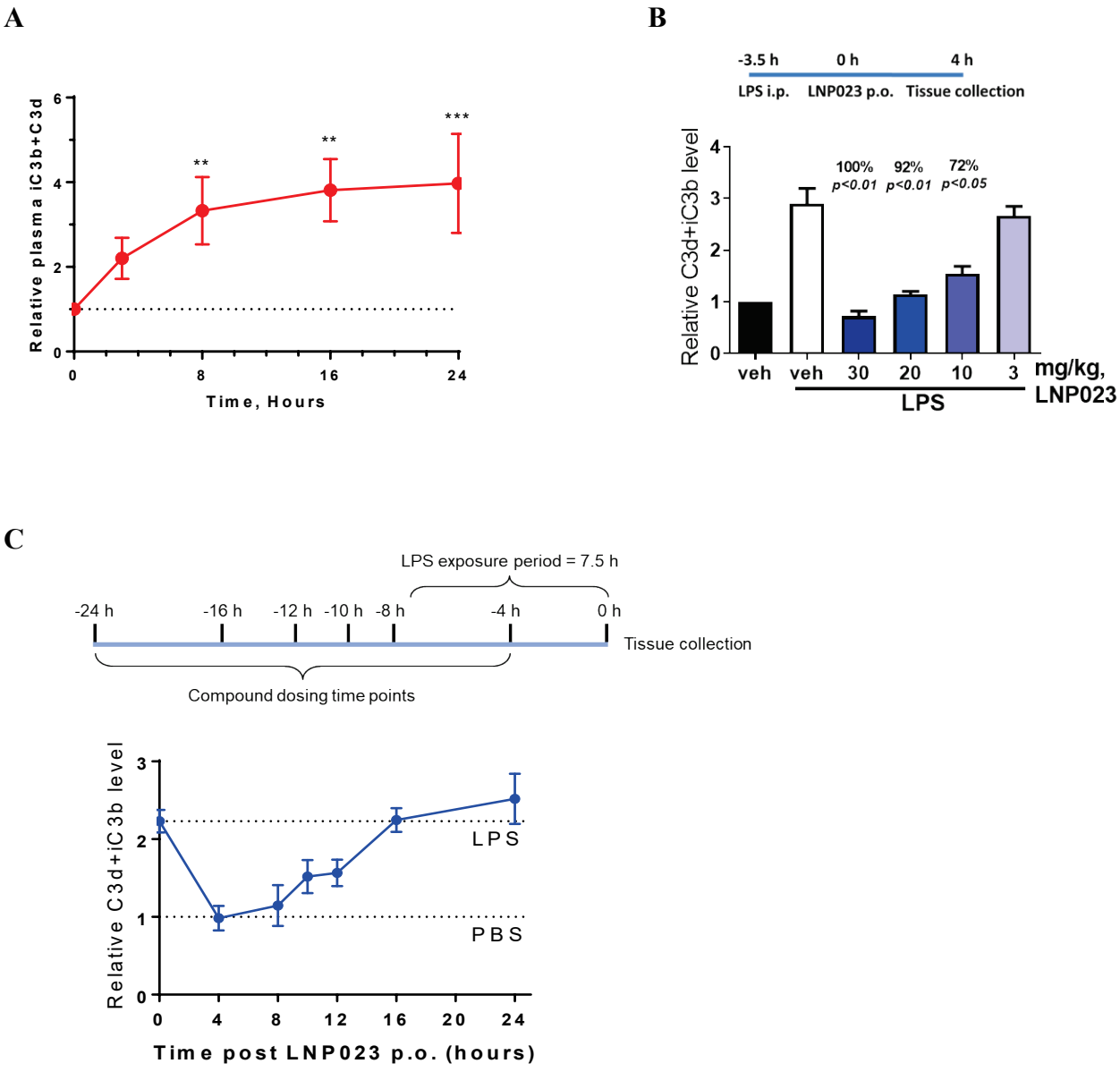
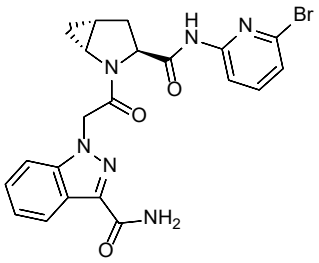


Fig. S5. *In vivo* inhibition of the AP by LNP023 after LPS challenge. (*A*) Injection of lipopolysaccharide (LPS) induces systemic AP activation in mice. Plasma C3d/iC3b levels after intraperitoneal injection of 50 µg LPS. Plasma of 7-week old female C57Bl/6J mice were collected at 0, 3, 7.5, 16, and 24 h after LPS injection and analyzed by semi-quantitative Western blotting. Levels in PBS treated mice were set at 1 (dashed line on graphs). Intraperitoneal injection of LPS induced a time-dependent increase of C3d/iC3b in plasma that was statistically significant by 7.5 h after LPS. At this time point, C3d/iC3b levels were 2 to 3-fold higher when compared to PBS-treated controls. Levels were similarly high at 24 h. Groups consisted of 4-6 mice. The group mean is presented, and error bars denote standard deviation. Differences were assessed by one-way ANOVA with Tukey's multiple comparison test, where **p<0.01, ***p<0.001 compared to the PBS

treated group. (*B*) Dose-dependent reduction of C3d/iC3b following administration of LNP023 by oral gavage at 3, 10, 20 and 30 mg/kg in mice 3.5 h after AP activation by intraperitoneal (ip) injection of LPS. Plasma was collected 4 h after dosing. The level of C3d/iC3b in the negative control groups (no LPS) was set at 1 (dashed line). Pos, positive control group. (*C*) Duration of effect of a single oral 30 mg/kg dose was examined using separate groups of mice that were administered LNP023 at 24, 16, 12, 10, 8, or 4 h prior to sample collection. The dosing scheme depicts the time points at which the single compound dose was given. AP activation by LPS was induced at 7.5 h prior to sample collection in all groups. The level of C3d/iC3b in the negative control group (no LPS) was set at 1, indicated by the line labeled PBS (saline). The level in the positive control group is indicated by the dotted line labeled LPS. Four female mice were used per group in all studies.



IC_{50} (FD) = 0.006 μM

IC_{50} (MAC, 50% serum) = 0.05 μM

Fig. S6. Chemical structure of the factor D inhibitor. The compound inhibits human factor D with an IC_{50} value of 0.006 μM and blocks zymosan-induced MAC formation in 50% human serum with an IC_{50} value of 0.05 μM.

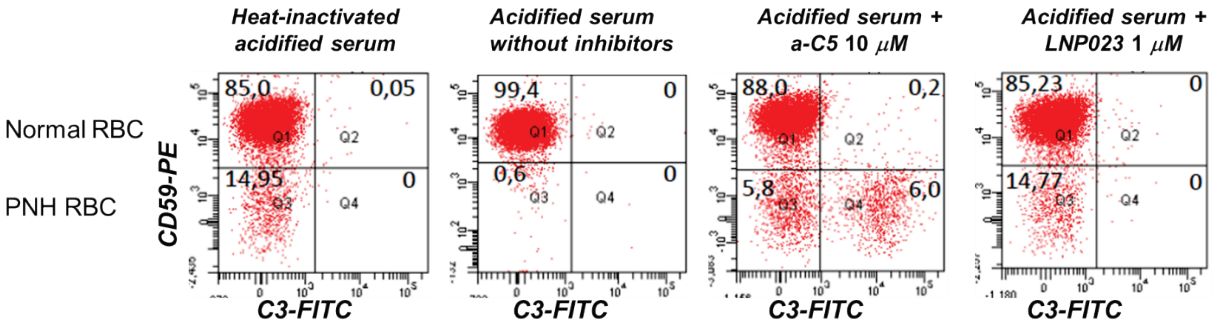


Fig. S7. LNP023 blocks hemolysis of and C3 deposition on PNH erythrocytes. Flow cytometry analysis after staining of the erythrocytes with a FITC-conjugated anti-C3 polyclonal and a PE-conjugated anti-CD59 monoclonal antibody. From left to right, in the presence of heat-inactivated serum (inhibition of all complement pathways), CD59-deficient red blood cells (RBCs) are present and not coated with C3 fragments; in the absence of inhibitors, CD59-negative RBCs are lysed in acidified serum; in the presence of anti-C5 antibody (a-C5), CD59-negative RBCs are spared from lysis, but are coated with C3 fragments; in the presence of 1 μM LNP023, CD59-negative cells are protected and not coated with C3 fragments.

Table S1. Small-molecule library and HTS screening data.

Category	Parameter	Description
Assay	Nature of the assay	Factor B DELFIA (dissociation-enhanced lanthanide fluorescent immunoassay)
	Primary measurement	Fluorescence measurement (serine protease activity) after compound incubation
	Reagents and sources	Complement Factors B, D, C3 (produced internally); CVF (Quidel Corp, CA, USA); Complete Protease Inhibitor Tablets (Roche Applied Sciences, CA, USA); Anti-C3b neoepitope antibody (Hycult Biotechnology, The Netherlands)
	Assay protocol	High throughput assay measuring Factor B activity, stabilized through the formation of CVF-Bb complex, and detection of C3a product for C3, using a specific monoclonal antibody
Library	Library size	~256,000
	Library composition	Diversity set
	Source	Novartis compound library
	Additional comments	Compound stocks at 2mM in 90% DMSO
Screen	Format	384-well
	Concentration(s) tested	30 μM
	Plate controls	No CVF-Bb enzyme; DMSO
	Reagent/ compound dispensing system	Thermo HTS-2 automated system
	Detection instrument and software	Envision (Perkin Elmer)
	Assay validation/QC	Average Z' values for the primary screen: 0.77
Post-HTS analysis	Hit criteria	Inhibition of $\geq$30%
	Hit rate	Overall <1%
	Structure confirmation	Compound structure verified by analytical chemistry methods

Table S2. Crystallographic data collection and refinement statistics

	Human FB – compound **1**	Human FB – compound **2**	Human FB – LNP023
PDB ID	6QSW	6QSX	6QSV
Data collection*			
Space group	P 4_32_12	$P2_1$	$P2_1$
Cell dimensions			
a, *b*, *c* (Å)	91.3, 91.3, 260.2	55.6, 98.1, 61.7	54.5, 97.4, 61.8
α, β, γ (°)	90, 90, 90	90, 99.2,90	90, 100.5, 90
Resolution (Å)	74.8-1.64 (1.70-1.64)	60.9-1.77 (1.83-177)	60-1.7 (1.74-1.7)
R_{sym} or R_{merge}	6.4 (36.2)	4.4 (27.9)	6.8 (81.4)
$I/\sigma I$	19.6 (2.5)	16.6 (4.25)	10.8 (1.8)
Completeness (%)	95.8 (76.6)	99.5 (99.9)	98.8 (96.6)
Redundancy	7.7 (2.6)	3.4 (3.5)	3.3 (3.1)
Refinement			
Resolution (Å)	74.7-1.64	51.8-1.77	48.7-1.7
No. reflections	129548	63252	68598
$R_{work/}$ R_{free}	15.7 / 18.8	18.9/22.0	19.5/22.7
No. atoms			
Protein	6280	4476	4467
Ligand/ion	54/50	44/35	62/27
Water	500	383	366
B-factors			
Protein	23.2	25.9	33.9
R.m.s deviations			
Bond lengths (Å)	0.01	0.01	0.01
Bond angles (°)	1.41	1.60	1.56

*Highest resolution shell is shown in parenthesis.

Table S3. Factor B proteolysis and AP complement deposition assay data.

	IC_{50} (μM)			
Compound	**Human FB inhibition**	**Human MAC deposition (50% serum)**	**Human MAC deposition (50% whole blood)**	**Mouse C3b deposition (50% serum)**
1	6.6 ± 3.5 (n=22)[a]	ND	35 ± 12 (n=2)	ND
2	3.5 ± 1.1 (n=4)	2.2 ± 1.1 (n=2)	24 ± 1 (n=2)	1.3 ± 0.14 (n=2)
LNP023	0.010 ± 0.006 (n=22)	0.13 ± 0.06 (n=6)	0.15 ± 0.02 (n=18)	0.11 ± 0.013 (n=4)

[a] n = number of experiments.
ND = not determined.
LNP023 showed no binding (IC_{50} values >100 μM) to the following human proteases *in vitro*, determined in biochemical assay formats at least in duplicates (n≥2), unless otherwise indicated: factor D, factor VIIa, factor IXa, factor Xa, plasma kallikrein, thrombin, plasmin, tPA, activated protein C (APC), urokinase, trypsin, chymase, chymotrypsin, membrane type serine protease 1, proteinase-3, tryptase-beta2, neutrophil elastase, pancreatic elastase, kallikrein 5, kallikrein 7, matriptase 2, caspase 1, caspase 3, caspase 8, cathepsin B, cathepsin G, cathepsin H, cathepsin K, cathepsin L (48 μM), cathepsin S (>30 μM), dipeptidyl-peptidase 2 (DPP2), DPP4, DPP8, DPP9, fibroblast activation protein-alpha, prolyl endopeptidase, matrix metalloproteinase 8 (MMP 8). LNP023 showed no binding (K_D values >30 μM) to the following G-protein coupled receptors, ion channels, receptors and transporters: serotonin 5HT1A, 5HT2A, 5HT2B, 5HT2C, 5HT3, 5HT4e, 5HT6 and 5HT7 receptor, adenosine 1, 2A and 3 receptor, adrenergic alpha 1A, 2A and 2B, alpha2C as well as beta1 and 2 receptor, androgen receptor, angiotensin II receptor, bradykinin 2 receptor, cholecystokinin alpha and beta receptor, cannibinoid-1 and 2 receptor, dopamine D1, D2 and D3 receptor, endothelin A receptor, estrogen receptor alpha, ghrelin receptor, glucocorticoid receptor, glucagon receptor, histamine H1, H2, H3 and H4 receptor, melanocortin MC1 and 3 receptor, melatonin 1 and 2 receptor, motilin receptor, muscarinic M1, M2 and M3 receptor, neurotensin receptor, NPY receptor, opioid delta and mu receptor, orexin 1 receptor, benzodiazepine receptor, GABA A receptor, nicotinic receptor, pregnane X receptor, progesterone receptor, prostacyclin receptor, prostaglandin F receptor, prostanoid EP-1, -2, -3 and -4 receptor, PPARgamma receptor, somatostatin 1 and 5 receptor, tachykinin receptor, thromboxane A2 receptor, thyroid hormone alpha and beta receptor, thyrotropin releasing hormone receptor, vasopressin 1A and 2 receptor, hERG-channel, adenosine transporter, dopamine transporter, 5-HTT transporter, monoamine transporter, norepinephrine transporter, and bile salt export pump (BSEP). LNP023 has IC_{50} values >30 μM for the following enzymes and kinases: Cox-1 and Cox-2, HER2/ErbB2 kinase, 5-lipoxygenase, MAO-A, PDE3, and PDE4D.

Table S4. Pharmacokinetic parameters of LNP023 in C57BL/6 mice.

Dose (mg/kg) iv / po	1 / 10
CL ($mL \cdot min^{-1} \cdot kg^{-1}$)	16
V_{ss} (L/kg)	6.6
$T_{1/2}$ / MRT (h)	7.2 / 1.6
$AUC_{0\text{-}7h}$ (nM·h), iv *	1657
$AUC_{0\text{-}7h}$ (nM·h), po *	760
C_{max} (nM) *	224
F (%)	46

*Values are dose normalized to 1 mg/kg.
Parameters are represented as a mean of two values (n = 2 per route of administration). Therefore, standard deviations have not been determined.
AUC, area under the curve; CL, clearance; Vss, volume of distribution; $T_{1/2}$, terminal half-life; MRT, mean residence time; F, absolute bioavailability.