SUPPORTING ONLINE MATERIAL:
Chemical induction of misfolded prion protein conformers in cell culture

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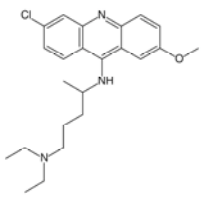
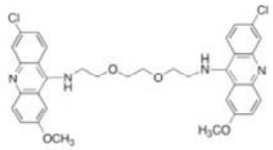
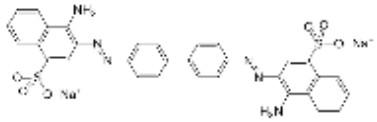
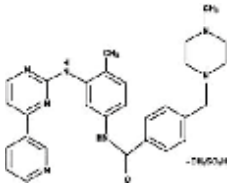
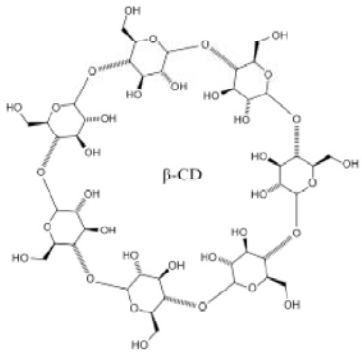
Table S1. Antiprion compounds assayed in this study.

Compound	Structure	Proposed mode of action	EC_{100}†	References
PAMAM-G4	See Fig. 3A	Direct interaction with PrP^{Sc}	10 µg/mL	(1,2)
Quinacrine		Direct interaction with PrP^{Sc}; disruption of lipid rafts	4 µM	(3,4)
Bis-acridine		Direct interaction with PrP^{Sc}; disruption of lipid rafts	1 µM	(5)
Congo red		Binding to amyloid	10 µM	(6)
D18	Fab fragment of anti-PrP IgG	Binding to PrP^{C}	10 µg/mL	(7)
Gleevec		Enhanced PrP^{Sc} lysosomal degradation	10 µM	(8)
MB-cyclodextrin		Disruption of lipid rafts	1.5 mM	(9)

† EC_{100} values refer to the lowest concentration that reduced PrP^{Sc} levels to below detection levels by Western immunoblotting of ScN2a-cl3 cells.

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SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. Total PrP and $rPrP^{Sc}$ levels in N2a-cl3 and ScN2a-cl3 cells. Cell lysates from uninfected N2a and N2a-cl3 as well as from RML-infected ScN2a and ScN2a-cl3 lines were collected from confluent cultures and the total protein concentration was determined by the BCA assay. RML samples were 10% brain homogenates from FVB mice infected with RML prions at the endpoint of disease; the brain homogenates were digested with PK and the final measurement was corrected with the appropriate dilution factor to determine the PrP level in 100% brain homogenate. For total PrP measurements, lysates were left untreated. For $rPrP^{Sc}$ measurements, 50 µg total protein was treated with PK (1:50 PK:protein). PrP levels were measured by ELISA and normalized to an absolute standard concentration ladder of recombinant full-length mouse PrP.

Fig. S2. The kinetics and dose-response profile of PK digestion of ScN2a-cl3 cells. ScN2a-cl3 lysates (50 µg total protein) were incubated with PK at **(A)** 1:50 PK:protein for the indicated durations or **(B)** the indicated PK:PrP ratios for 60 min. Incubations were performed at 37 °C. The population of undigested PrP^{Sc} for each time point was measured by ELISA. The error bars indicate the standard deviation of three replicate measurements.

Fig. S3. The effect of sodium butyrate on the growth and $rPrP^{Sc}$ levels in ScN2a-cl3 cells. **(A)** Sodium butyrate halted growth of ScN2a-cl3 cells. A confluent culture of ScN2a-cl3 cells was split by a factor of 1:10 into multiple 10-cm plates. After the indicated number of days, the cells were dissociated and the cell number was

determined by a hemacytometer. After 3 days of growth, 10 mM sodium butyrate was added to the experimental arm (indicated by the arrow); the control arm was left untreated. Cell numbers were counted each day for the next 4 days. **(B)** Sodium butyrate did not affect $rPrP^{Sc}$ levels in ScN2a-cl3 cells. To 30% confluent ScN2a-cl3 cells, 10 mM sodium butyrate was added and incubated for 96 h (fresh media and sodium butyrate was added after 48 h). The cells were lysed and normalized to 1 mg/mL total protein concentration. The levels of total PrP (–PK, black bars) and $rPrP^{Sc}$ (+PK, white bars) were quantified by ELISA.

Fig. S4. PAMAM treatment did not affect *Prnp* mRNA expression levels. To N2a-cl3 and ScN2a-cl3 cells, 25 µg/mL PAMAM was incubated for 3 days, after which relative *Prnp* mRNA levels were determined (black bars). Untreated cells (white bars) are shown as a control.

Fig. S5. Induction of insoluble PrP aggregates by PAMAM-G4 in N2a-cl3, N2a, and GT1 cells. Cells were grown to 80% confluency before the addition of 10 µg/mL of PAMAM-G4 for 20 h. Lysates were divided into two aliquots; one aliquot was digested with PK (to determine insoluble, protease-resistant PrP levels) and the other aliquot was left undigested (to determine total insoluble PrP levels). All samples were PTA precipitated. Western immunoblotting was performed using D13 Ab. Apparent molecular masses based on the migration of protein standards are shown in kilodaltons.

Fig. S6. The epitopes of the four PrP antibodies utilized in this study. Blue indicates the N-terminal ER translocation and signal sequences for the addition of the glycosylphosphatidyl inositol anchor. Orange indicates the unstructured N-terminal

domain. The arrows and cylinders indicate β-sheet and α-helical structures, respectively, in PrP^C (based on structure 1QLX in PDB). EST123, D13 and D18 can detect the wt mouse PrP sequence. Ab 3F4 can only detect the mouse PrP sequence containing the L109M and V112M mutations, as expressed in MHM2.

Fig. S7. Cellular localization of PrP^A. N2a-cl3 cells were plated on a poly-lysine D–coated coverslip, then left untreated or treated with 10 ng/ml of PAMAM-G7 or PAMAM-G5.5 for 24 h. The cells were fixed with 4% paraformaldehyde, permeablized with 0.3% TX-100 for 5 min and treated with 3 M GdnSCN for 8 min prior to blocking with 10% normal goat serum (NGS). Cells were stained with either anti-PrP D18 or anti-actin primary antibody and fluorescence-conjugated secondary antibodies. Cells were visualized with a fluorescence **(A)** or confocal **(B)** microscope, as described in *Experimental Procedures*.

Fig. S8. The interaction of immobilized PAMAM-G7 with PrP. Sepharose 4B beads were coupled to PAMAM-G7 or were blocked without coupling. Beads (100 uL) were added to 1 mL of 10% brain homogenates from uninfected FVB mice and incubated for 1 h at room temperature with rotation. After the beads were removed by centrifugation, the homogenates were either run directly (Total) or precipitated with PTA then run (PTA pellet) on Western blots. Western immunoblotting was performed using D13 Ab. Apparent molecular masses based on the migration of protein standards are shown in kilodaltons.