Supporting Information

Resolution of Omeprazole Using Coupled Preferential Crystallization: Efficient Separation of a Non-Racemizable Conglomerate Salt under Near-Equilibrium Conditions.

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General Methods.

Racemic omeprazole (95%) and (*S*)-1,1,2-triphenyl-1,2-ethanediol (95%) were purchased from TCI Chemicals America. Ethanol (Acros organics, 99.5% ACS grade), water (Fisher scientific, HPLC grade) and toluene (Fisher Scientific, ACS grade) were used as received without further purification. Samples of *S*-omeprazole were obtained by selective crystallization as a distereomeric complex as outlined below.

Enantiomeric excess of the omeprazole crystal phase was monitored using high pressure liquid chromatography – mass spectrometry (HPLC-MS), carried out using on an Agilent 1100 coupled to an SL ESI single quad MSD. Separation of enantiomers was accomplished using a Chiracel® OJ-RH reverse phase chiral column (mobile phase – EtOH, absorbance @ 235nm, column specs - 4.6mm ID x 250 mm, 5µ pore size, Chiral Technologies).

**Preparation of Omeprazole-mono potassium/ethanol solvate**
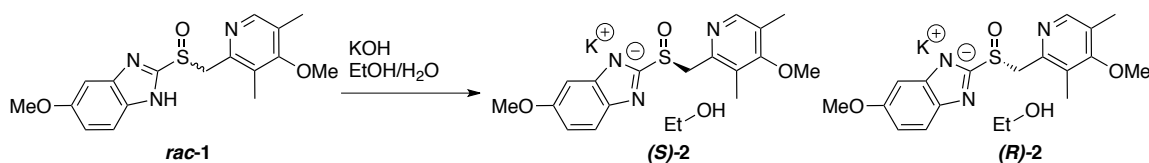
Scheme S1: Crystallization of Omeprazole as a racemic mixture of conglomerate crystals.

Conglomerate crystals of *S*- and *R*-**2** were prepared via a procedure modified from Coquerel et al.[1] Rac-omeprazole (4.0 g, 11.6 mmol) was suspended in 5% water in ethanol (100 mL) at 25°C. KOH (1.212 g, 21.6 mmol) was added and the sample was stirred at high speed until a homogenous solution was obtained. The solution was then covered with parafilm and set undisturbed in a freezer set to -10°C until crystals formed. These crystals were isolated by filtration to produce *S*- and *R*-**2** (4.1 g, 9.5 mmol 82%). Freshly isolated crystals were used immediately in the resolution apparatus. Allowing the crystals to stand open to the air or even in a

sealed vial led to rapid change in both consistency and texture. We attribute this to loss of the volatile ethanol from the solvate crystal structure.

Isolation *S*-Omeprazole-mono potassium/ethanol solvate.
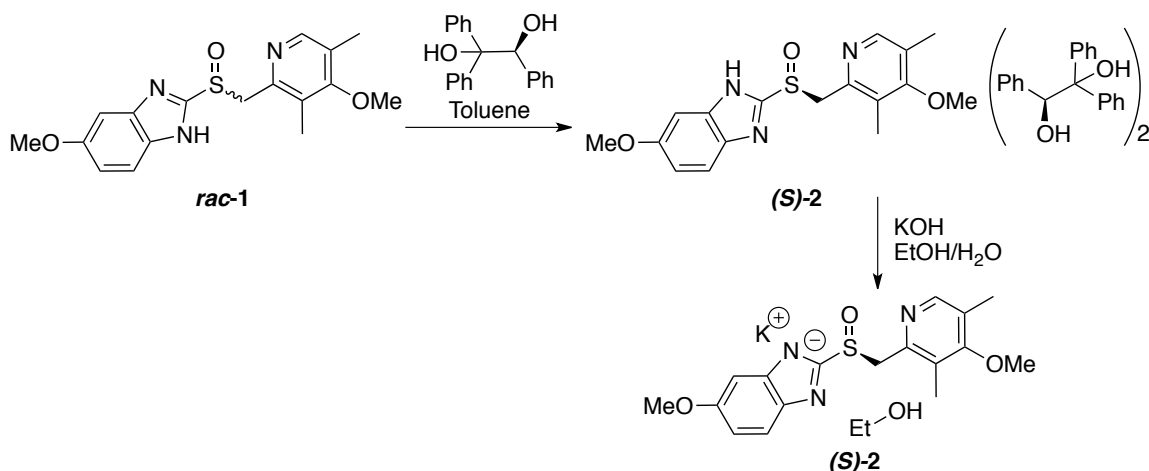
Scheme S2: Crystallization as diasteromeric inclusion complex followed by generation of enantiopure potassium salt/ethanol solvate.

Enantiopure S-**2** was obtained by a two step procedure modified from Coto et al.[2]

1) rac-omeprazole (5 g, 14.5 mmol) was suspended in toluene (220 mL) and heated to 80°C. At this temperature (*S*)-1,1,2-triphenyl-1,2-ethanediol (6.3 g, 21.7 mmol) was added to produce a darkly colored homogenous solution. The solution was cooled slowly to 25°C over the course of 1 hour, over which time a white crystalline precipitate formed. The slurry was stirred at 25°C for an additional hour and then the precipitate was isolated by filtration. The crystals were washed with toluene (3×25 mL) and the dried under vacuum to produce (S)-omeprazole.2-[(S)-1,1,2-triphenyl-1,2-ethanediol] inclusion complex (5.8 g, 6.3 mmol, 86%, e.e. 98%)

2) (S)-omeprazole.2-[(S)-1,1,2-triphenyl-1,2-ethanediol] inclusion complex (1.17 g, 1.26 mmol) was dissolved in 5% water in ethanol (6.5 mL) at 35°C. The solution was treated with KOH (0.128 g, 2.28 mmol) and then set in the freezer to cool to -10°C until crystals formed. These crystals were isolated by filtration and then recrystallized from 5% water in ethanol (~4 ml) containing KOH (0.05g,) to obtain pure (S)-**2** (0.48g, 1.11 mmol, 88%, 95% e.e.).

HPLC-UV Chromatograms separated on Chiracel OJ-RH column – EtOH mobile phase
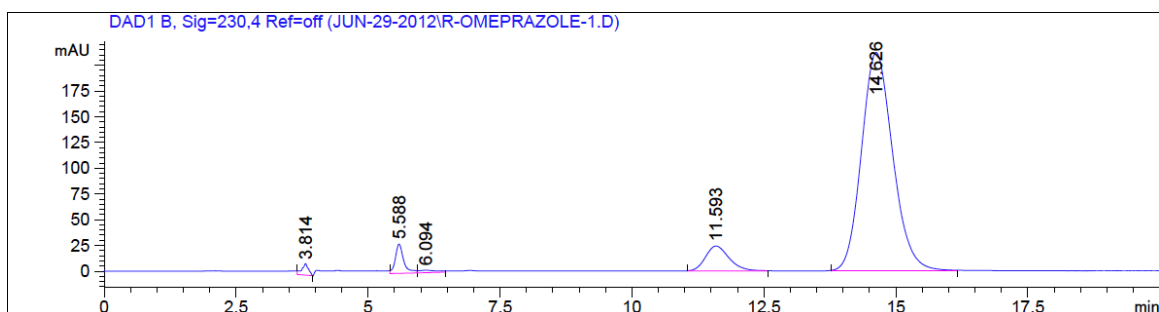
Racemic Omeprazole
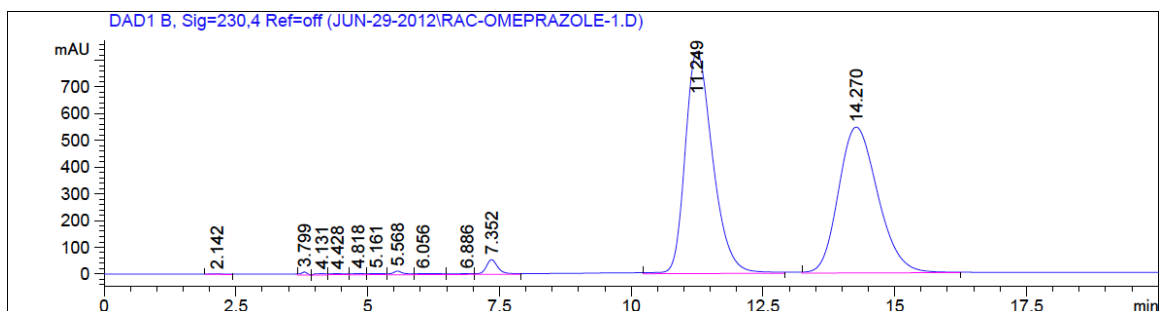
S-Omeprazole
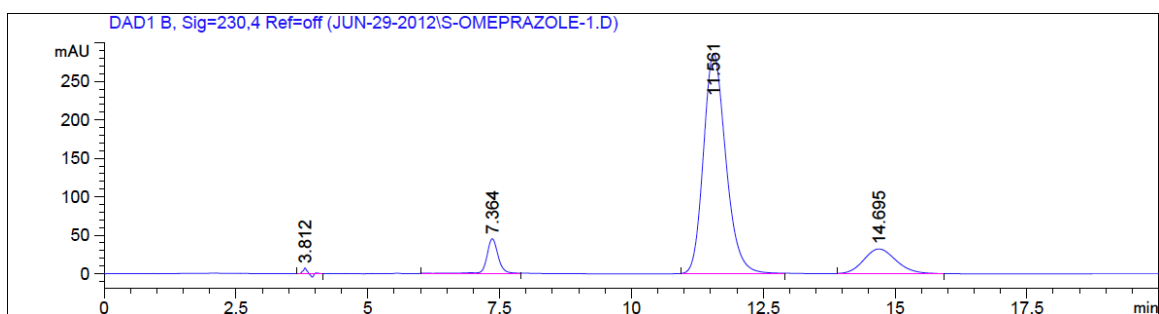
R-Omeprazole

Calculation of solubility and estimation of the metastability region by in-situ ATR-FTIR

In situ IR spectroscopy was performed using a Mettler-Tolledo ReactIR-15 equipped with a 6.3mm AgX DiComp fiberoptic probe. All crystallization and solubility experiments were conducted in a Mettler-Tolledo EasyMax lab reactor,

which allows precise and accurate control of solution temperature and stir rate.

    a) ***calibration curve***: a flask containing 5% water in ethanol was thermostated to 25°C in the EasyMax. Portions of *rac*-2 were then added while the IR spectra was recorded. The peak height at 1155cm^{-1} (relative to a two point baseline) was used to create a linear calibration curve in order to quantify the solution phase concentration of omeprazole.
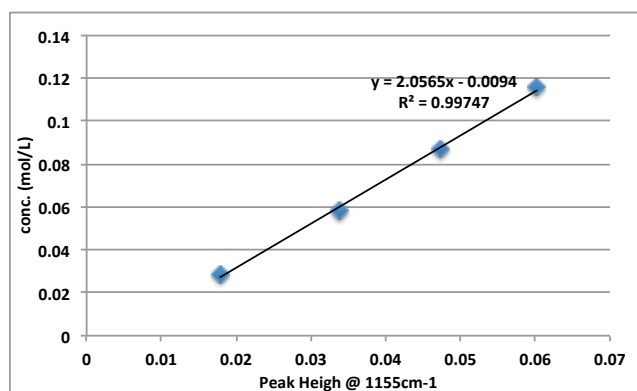

Figure S1: Calibration of IR response fro solutions of rac-omperazole

    b) ***Solubility measurements***: a flask containing 5% water in ethanol (50mL) was thermostated to 0°C in the EasyMax and monitored by the IR probe. The flask was charged with *rac*-**2** (4.98 g, 11.6 mmol) and the resulting slurry was stirred at 600 RPM. Once the peak height at 1155cm^{-1} (relative to a two point baseline) had stabilized the temperature was increased by 5°C and process was repeated to create a solubility profile for *rac*-**2** over a temperature range of 0 – 35°C. Important note: at all temperature points the system remained saturated and a stabile crystalline phase was present.

    c) ***Measurement of primary nucleation***: a flask containing 5% water in ethanol (50mL) was thermostated to 35°C in the EasyMax and monitored by the IR probe. The flask was charged with *rac*-**2** (1.77 g, 4.11 mmol) and stirred using a magnetic stir bar (Belart Scienceware, 25.4mm X 25.4mm, Teflon coated cross) at a rate of 150 RPM to give a homogenous solution. The solution was then cooled at a rate of 1°C per minute while stirring at 150 RPM, while the peak height at 1155cm^{-1} (relative to a two

point baseline) was monitored. The cooling profile was continued until nucleation and crystallization occurred (as indicated by a sudden drop in the IR signal). The flask was warmed back to 35°C to regenerate a homogenous solution, then the cooling profile was repeated twice more and the temperature for primary nucleation for the three experiments were averaged. Once this series was complete the concentration of the system was increased by introducing addition *rac*-**2**. The solution was heated until it became homogenous and then the cooling experiment was repeated.
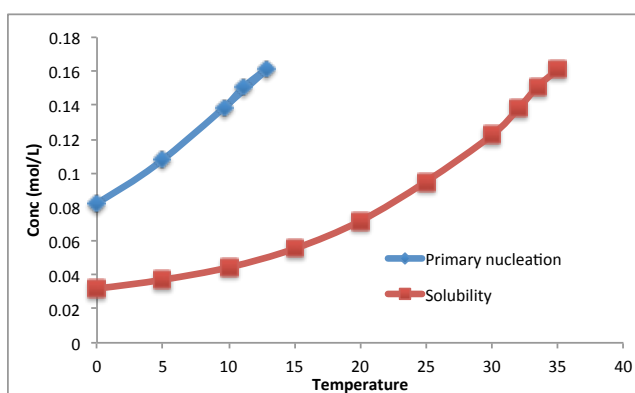

Figure S2: Solubility and primary nucleation limits for *rac*-**2**

Attempt to isolate (S)-2 by classical preferential crystallization

Some conglomerate crystalline materials are poor candidates for resolution by classical preferential crystallization, due to a high propensity for primary nucleation of the unseeded (or undesired) enantiomer during the cooling phase. This limitation was found to be a significant problem with racemic mixtures of conglomerate (*S*)-**2** and (*R*)-**2**. This was illustrated by an experiment where racemic **1** was dissolved in EtOH/water, treated with KOH and heated to 35°C to create a homogenous solution. The sample was then cooled to 25°C over 100 minutes while monitoring by ReactIR. The invariance in solution phase concentration confirms that no primary nucleation events took place (Figure S3, before point **A**). Seed crystals of *(S)*-**2** were then added (Figure S3, point **A**), which initiated crystallization leading to a progressive drop in solution phase concentration (Figure S3, point **B**). Crystals collected during this period contained only *(S)*-**2**, however, at some point

the rate of crystallization suddenly increased (Figure S3, inflection point **C**). This event correlates to a drop in the crystal phase enantiomeric excess, confirming that nucleation of *(R)*-**2** had occurred. Crystallization of both enantiomers continued until the solid phase is racemic and the system had reached concentration equilibrium at 25°C (Figure S3, point **D**).
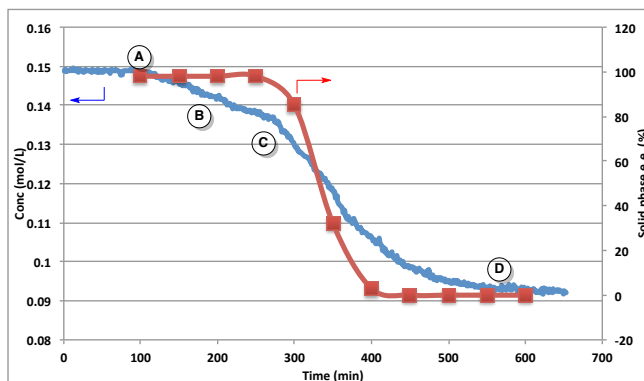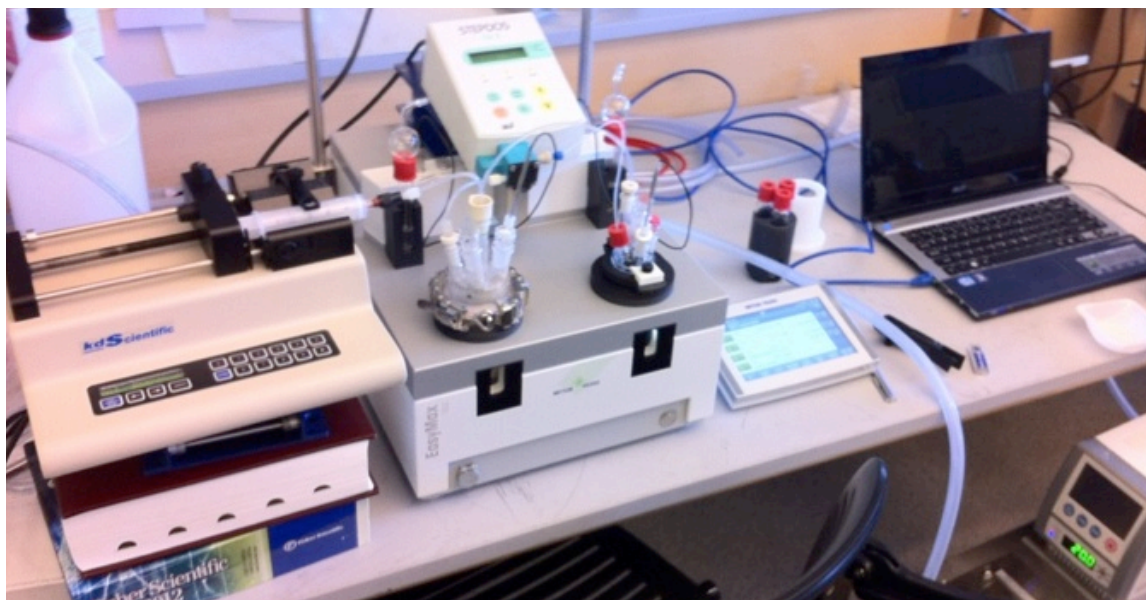

Figure S3: Solution phase concentration and solid phase enantiomeric excess as a function of time.

Apparatus for Coupled Preferential Crystallization

The system used to carry out two-pot crystallizations was composed of

1) EasyMax Synthesis Workstation (Mettler-Tolledo AutoChem)
2) React-IR iC10 with AgX fibercable and DiComp probe (Mettler-Tolledo

AutoChem)

3) Stepdos 03 S diaphragm metering pump (KNF Lab)

4) Standard 1/8" OD tubing fitted with in-line mobile phase filters (2 μm sintered stainless steel – Upchuch Scientific)

Failure of resolution by attrition-induced solubility gradient

All attempts to separate mixtures of (S)- and (R)-**2** using grinding-induced solubility differences failed. Grinding a racemic mixture of **2** led to complete ablation of the solid phase within the attrition vessel with concomitant growth of both (S)- and (R)-**2** in the receiving vessel. We attribute this result to a greater than expected solubility increase of conglomerate **2** upon grinding, possibly due to the formation of an amorphous solid phase upon mechanical ablation. In part, this hypothesis is supported by *in situ* ATR-FTIR measurements, which show a gradual increase in solution phase concentration during grinding (Figure S4, point A). This increase in solution concentration continues until there is an abrupt drop (Figure S4, point B). Measures of the crystal phase in the receiving flask confirms that a single enantiomer persists up until this sudden solution phase drop, after which the unseeded enantiomer is also present, conforming primary nucleation and crystal growth has occurred.
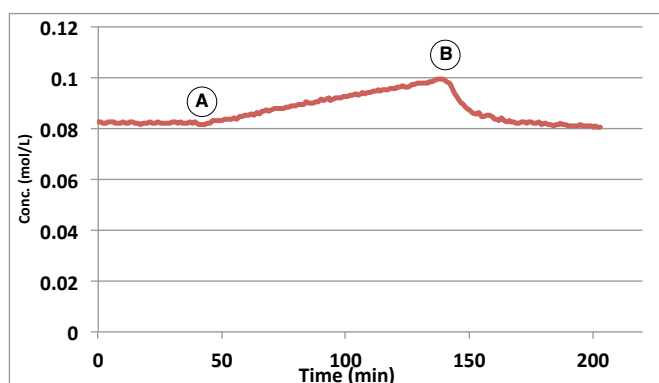

Figure S4: Solution phase concentration of omeprazole during attempts to resolve racemic crystals using grinding.

Resolution of *R*- and S-2 using coupled preferential crystallization.

A saturated solution of omeprazole was created by adding *rac*-**2** (5.21g, 12.12 mmol) to 5% water in ethanol (150 mL) and stirred at 21°C for 30 min. The slurry was filtered to give a clear filtrate, which was charged into flask A and B (~50mL in each) of the EasyMax (T = 21°C). Flask A was then charged with *rac*-**2** (4.50 g, 10.47 mmol) while flask B was seeded with *S*-**2** (0.10g, 0.02 mmol). Each flask was stirred at 150 RPM for 10 minutes, with the internal temperature set to 21°C. Flask A and B were then connected sequentially to two separate Stepdos 03 S diaphragm metering pumps using Teflon® PFA tubing (1/8 OD) fitted on the uptake with a fritted stainless steel filter (2 μm porosity). The crystal-free solution phase was circulated between flask A and B at a rate of 2 mL per minute. Once the flow rate was stabile the flask A was increased to 25°C. The solution phase circulated at 2 mL per minute for 24 hours. At regular intervals samples of the slurry from flask A and B were withdrawn and the crystals isolated by filtration and checked by chiral HPLC. Crystal phase e.e. from flask B was consistently >98% *S*-**2**, while the solid phase in flask A progressively showed an enrichment in *R*-**2**.

Impact of temperature bias on resolution.

Although the effect of the temperature difference between flasks was not studied exhaustively, it is obviously a critical factor for the resolution. To illustrate this a series of resolutions were carried out by charging the source flask (flask A) with 6g of a racemic mixture of **2** and 50mL of 5% water in ethanol. The liquid phase was circulated between flask A and flask B (both flasks set to T = 21°C) at a rate of 0.5mL per minute (stir rate for both flasks = 600 RPM). During this time no nucleation or crystal growth was observed in flask B. Seed crystals of (S)-2 (~0.25 g) were then added to flask B and the liquid phase was allowed to circulate to allow the system to equilibrate. After ~10 minutes the temperature of flask A was raised to T_1 (Figure S5) at a rate of 0.1°C per minute. The solution phase was allowed to circulate at flow rate of 0.5mL/min for 12 hrs. The crystal phase (if present) from

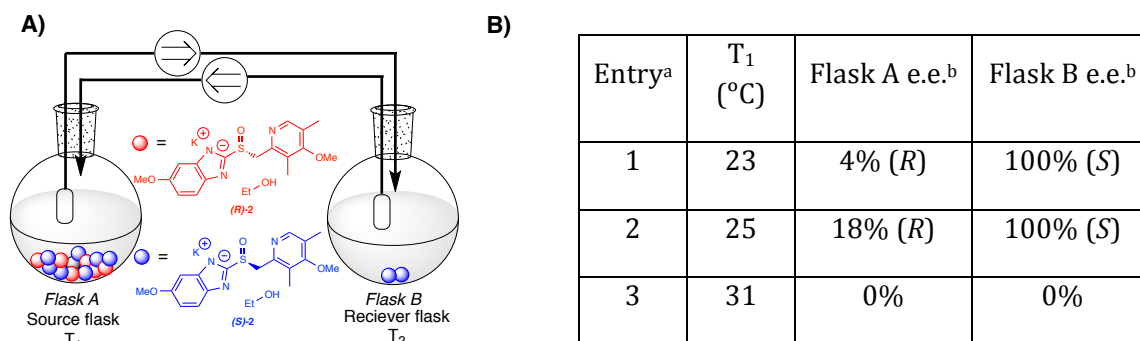flask A and B were independently sampled.

A)

B)

Entry[a]	T_1 (°C)	Flask A e.e.[b]	Flask B e.e.[b]
1	23	4% (*R*)	100% (*S*)
2	25	18% (*R*)	100% (*S*)
3	31	0%	0%

Figure S5: Coupled preferential crystallization using temperature-bias, [a]T_2 = 21°C for all entries; [b]e.e. of isolated crystals taken after 12 hours circulating.

This experiment illustrates that the rate of separation varies with the magnitude of the temperature bias (Figure S5B, entry 1 vs 2). After an equivalent time less mass transfer has occurred with a temperature difference of only 2°C (given by the small solid phase e.e. in flask A) while no primary nucleation of has taken place in flask B. However, a maximum threshold exists, beyond which primary nucleation of (*R*)-**2** in flask B becomes likely due to a very large concentration gradient (Figure S5b, entry 3)

[1] G. Coquerel, M.-N. Noelle, US 2009/0124811 A1, **2009**.

[2] A.D. Coto, A. Comely, X.V. Espaulella, L.R. Jane, US 2008/0300411 A1, **2008.**