Synthesis of (−)-PNU-286607 by Asymmetric Cyclization of Alkylidene Barbiturates

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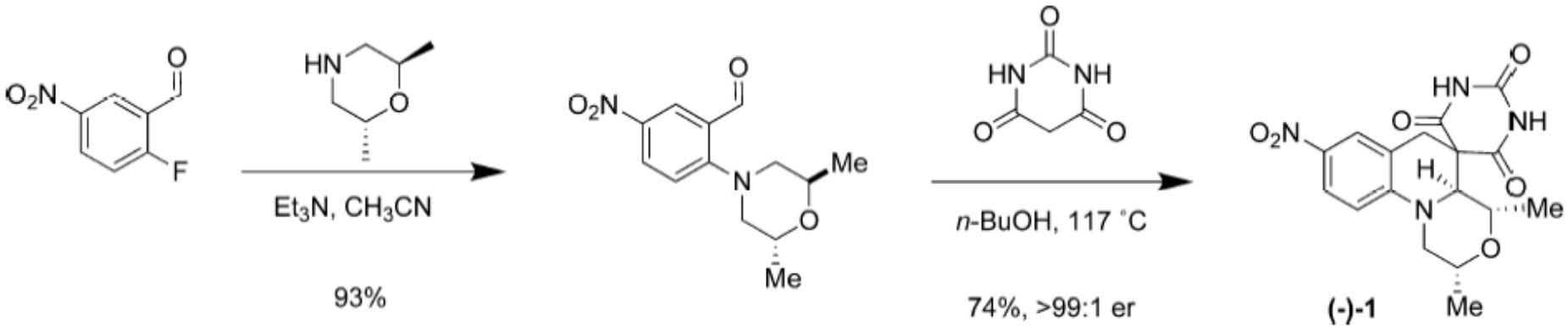

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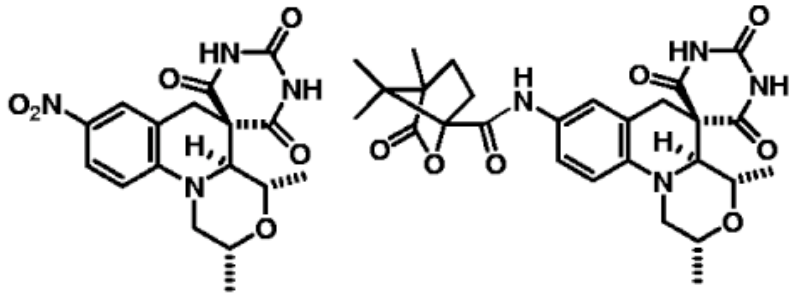
Characterics of PNU-286607

- PNU-286607 was identified during a screening effort at Pharmacia and Upjohn for compounds possessing **whole cell antibacterial activity** out of ~250,000 compounds.

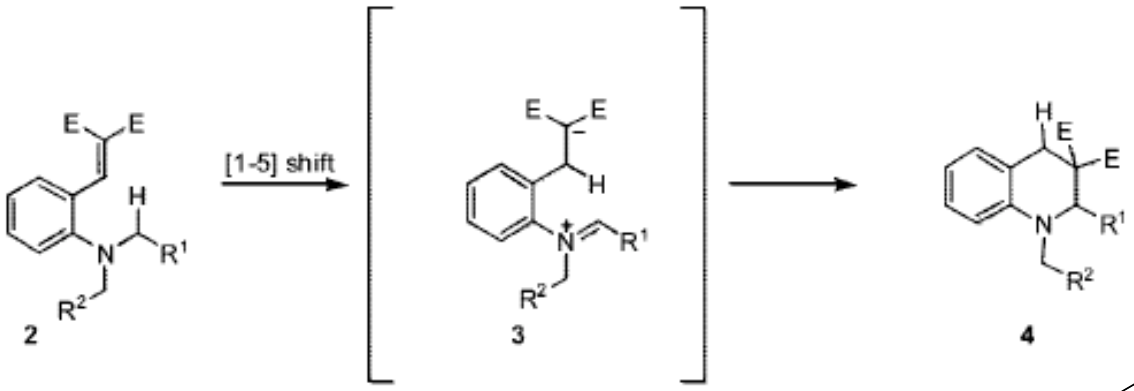
- A **reverse chemical genomics approach** (compound-driven target identification) led to the identification of **bacterial type II topoisomerase enzymes** (DNA gyrase and topoisomerase IV) which are essential for bacterial DNA synthesis. PNU-286607 targets these enzymes via a mechanism of inhibition distinct from the mechanisms of fluoroquinolones and novobiocin. Further, PNU-286607 displays **little cross-resistance with marketed antibacterial therapies**. Since there is a dire need for new molecular entities and antibacterial agents with novel mechanisms of action to counter bacterial resistance, PNU-286607 represents a promising opportunity.

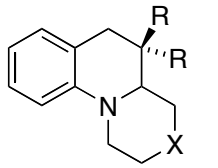
- Upon attempts to resynthesize the originally assigned structure of PNU-286607, it became clear that the structure was incorrect. Extensive NMR studies and an X-ray crystal structure determination revealed that PNU-286607 had the structure shown in Figure 1.



- PNU-286607 presented several significant synthetic challenges due to its structural complexity. In addition to the **unusual spirocyclic barbituric acid moiety**, the compound possesses **three stereogenic centers around the fused morpholine portion** of the tetrahydroquinoline core. Diastereocontrol of these stereogenic centers was of particular concern. Further, since the antibacterial activity resides solely in the (-)-enantiomer of PNU-286607, an **asymmetric synthesis** was given high priority.

tert-Amino Effect/Cyclizations



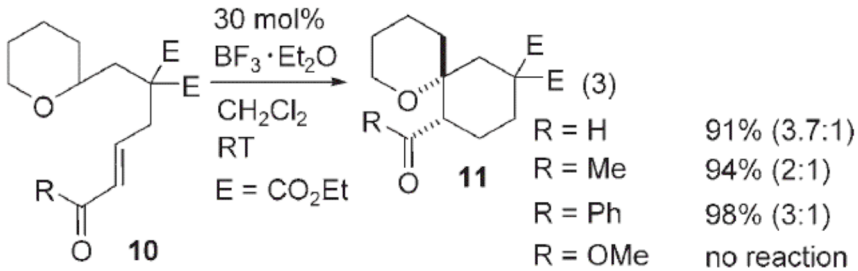


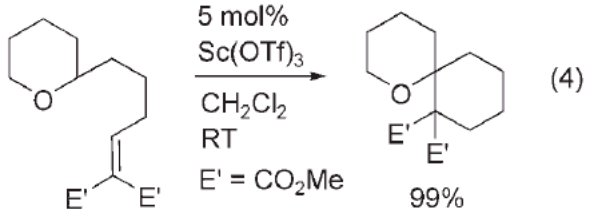
R = E or CN
X = -
CH_2
O
NR

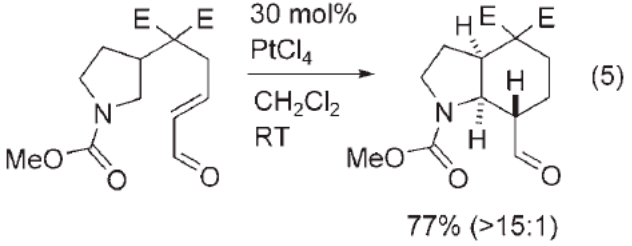
1) Meth-Cohen, O.; Suschitzky, H. *Adv. Heterocyl. Chem.* **1972**, *14*, 211.

2) Reinhoudt *et. al. JOC* **1984**, *49*, 269: *Synthesis* **1987**, *7*, 641: *Tetrahedron* **1988**, *44*, 4637: *JOC* **1989**, *54*, 199: *JOC* **1989**, *54*, 209.

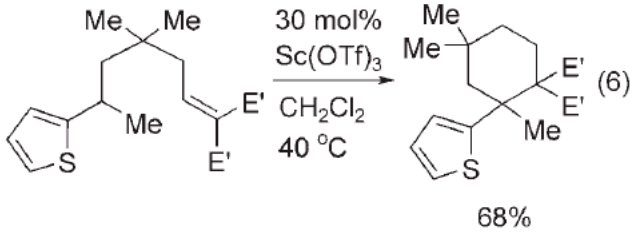
3) Pastine, S. J.; McQuaid, K. M.; Sames, D. *JACS* **2005**, *127*, 12180.





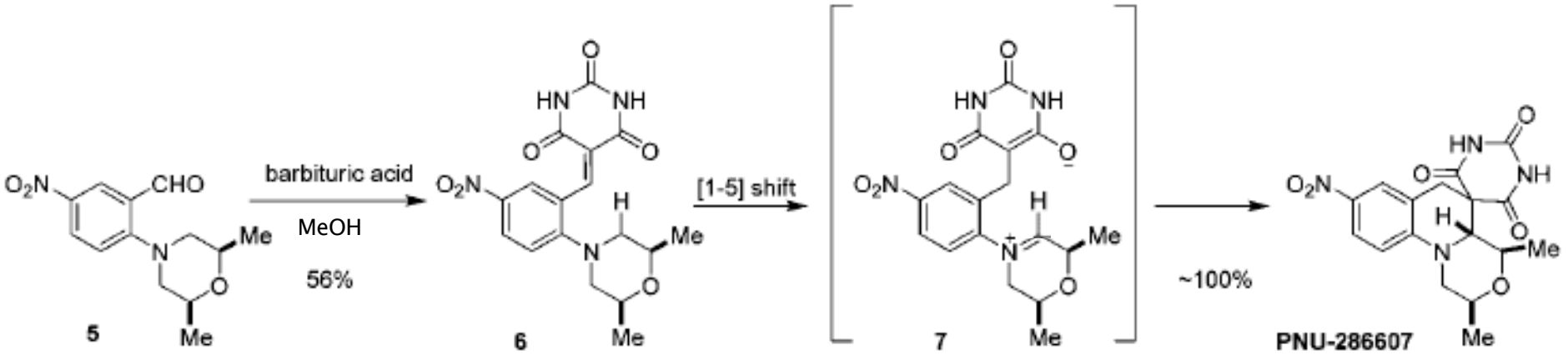


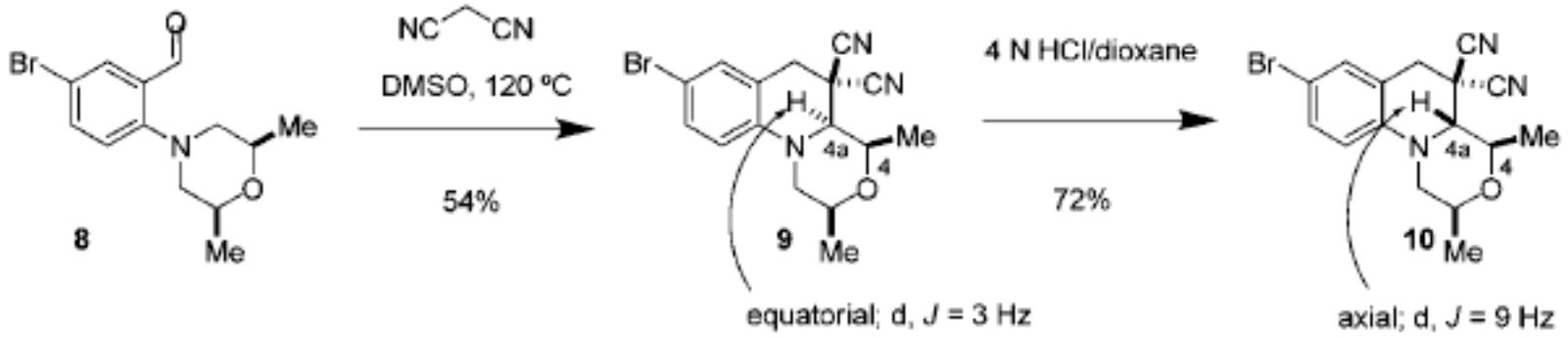
- **Functionalization of sterically hindered 2°, 3°, and benzylic C-H bonds**
- **12 additional examples: 68 99% yield**



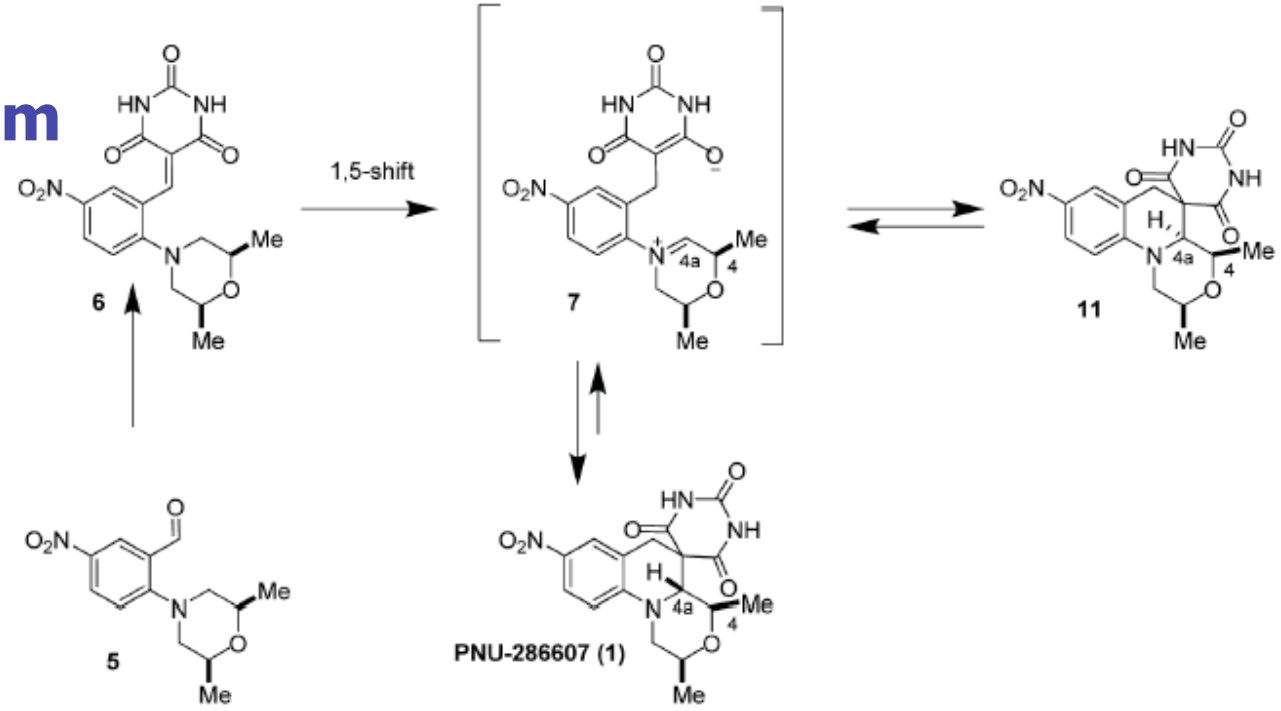
Preliminary Synthesis of PNU-286607

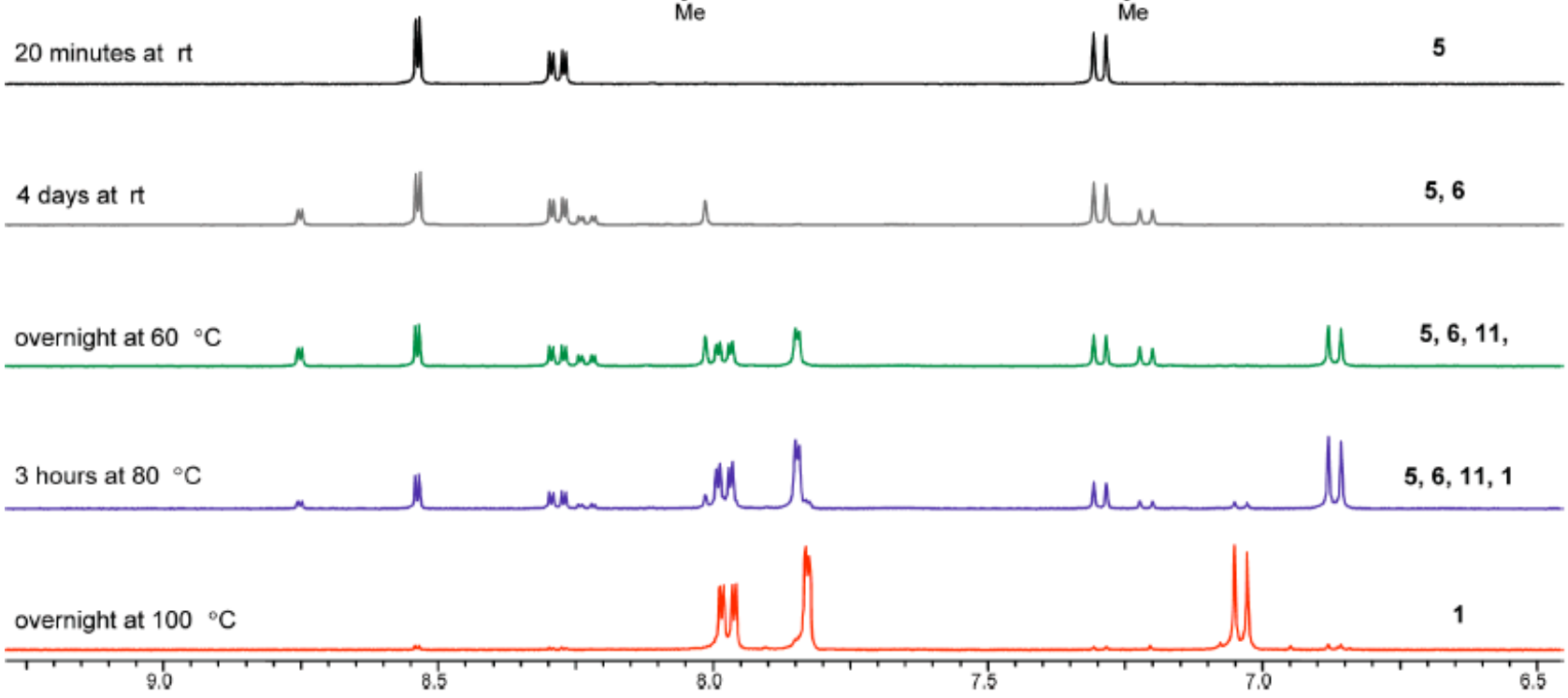
- There were very few diastereoselective tert-amino reactions wherein the resident stereogenic centers were located on the pendant azacycle, and none with a stereogenic center located adjacent to the migrating hydrogen.





NMR Study for Synthetic Mechanism of PNU-286607 in DMSO-d_6

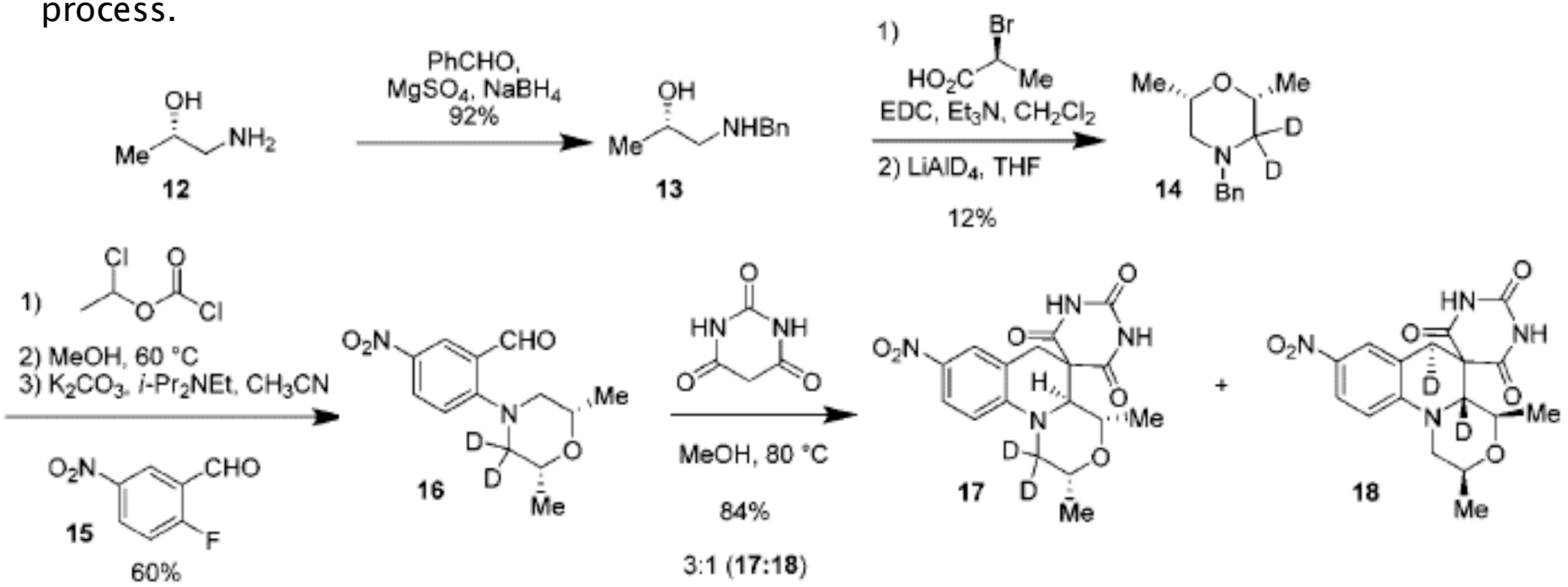




Study for [1,5]-shift

- Reinhoudt and co-workers have investigated the [1,5]-shift with pyrrolidine-2,2,5,5-d_4, determining the **isotope effect of 3.0 ± 0.3** at 91.2 °C in DMSO-d_6. This result significantly supports **[1,5]-shift is the rate-determining step**. —*Tetrahedron* **1988**, *44*, 4637.

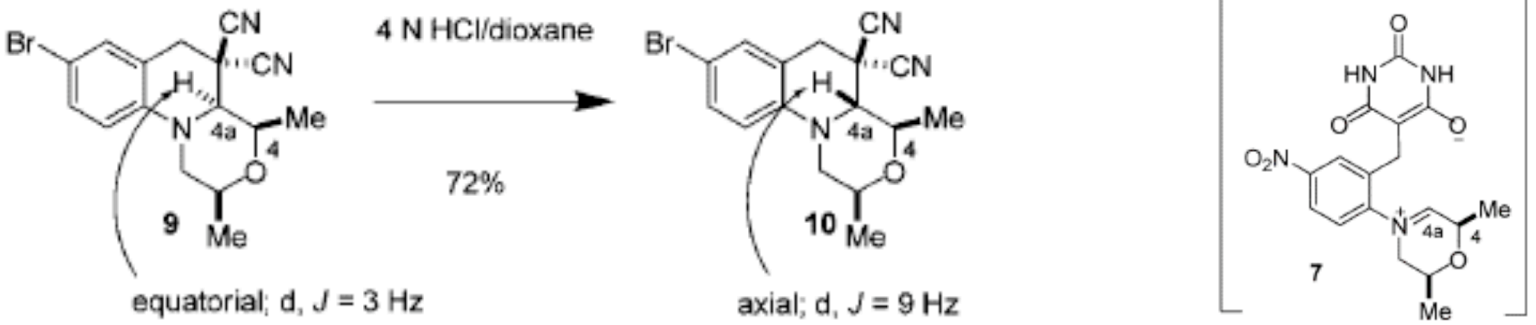
- Authors were interested in studying a **nonsymmetrically labeled morpholine** for deeper mechanistic insight and ultimately to examine the possibility of developing an asymmetric process.



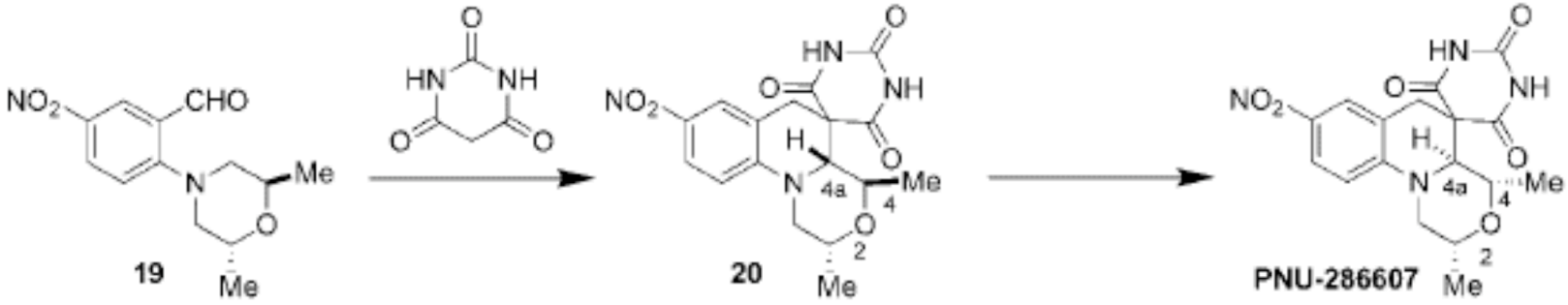
- Deuterium migration was stereoselective in compound **18**. The ratio of **17** to **18** was ~3:1, in accordance with the reported isotope effect. Products derived from double deuterium migration were not detected, which indicates that **1,5-shift might not be reversible**.

- First asymmetric synthesis of deuterated **17** reconfirmed the absolute stereochemistry and **17** has shown the equal antibacterial activity as (-)-PNU-286607; **18** was devoid of activity.

Study for Stereoselective Cyclization



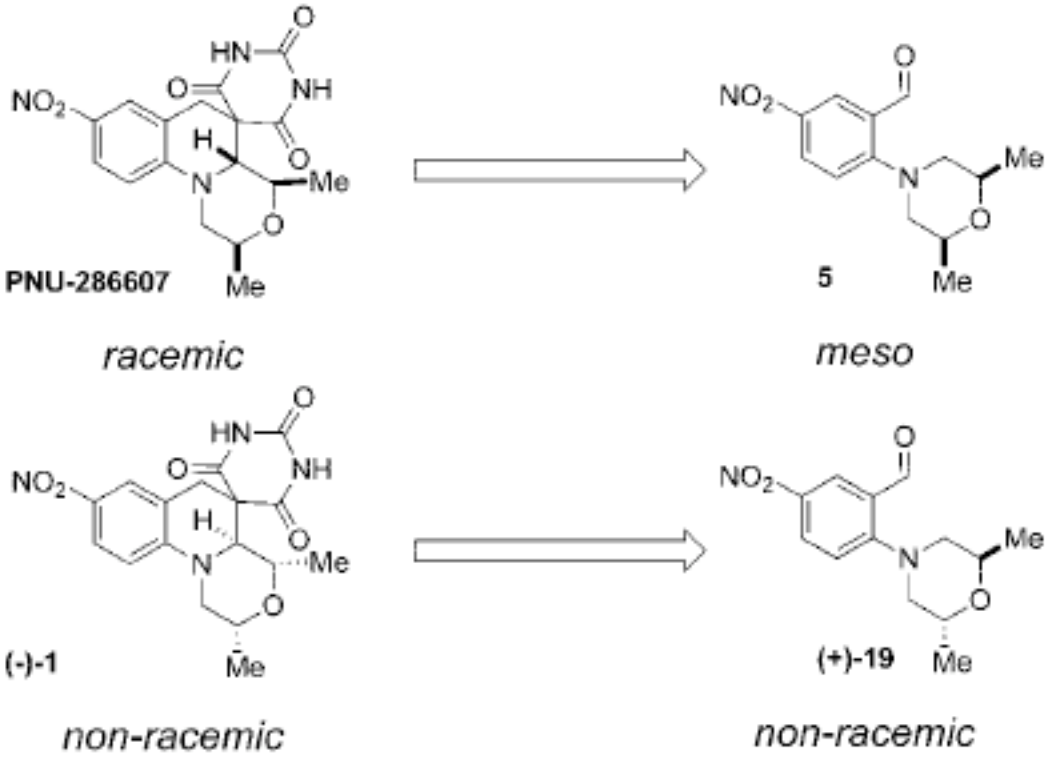
- C(4a) was stereochemically labile dependent on the configuration of adjacent methyl group on C(4). However, examination of the C(4a) isomerization mechanism led authors to consider whether the C(4) methyl-bearing stereocenter was labile as well, via the putative iminium ion intermediate **7**.

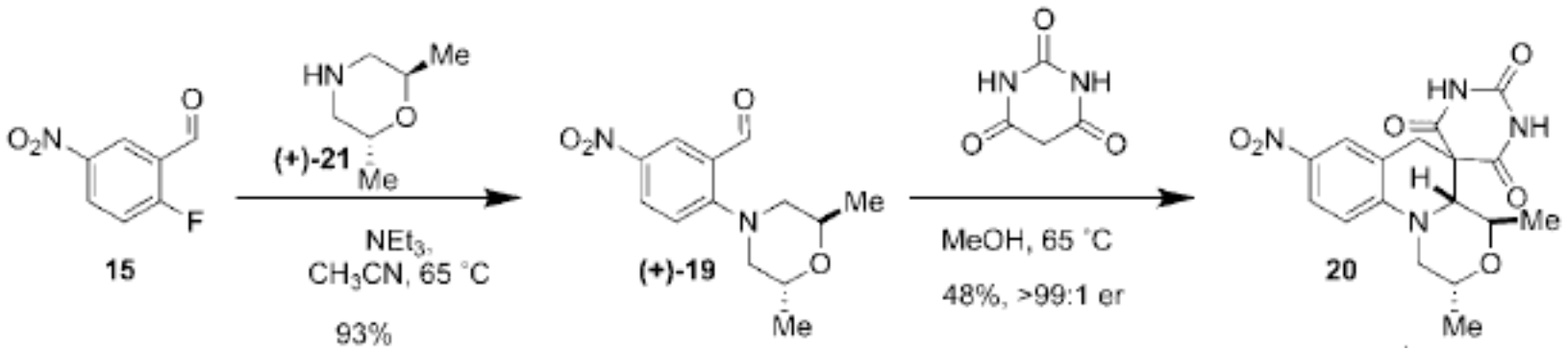


- A small amount of the cis-product (PNU-286607) was observed during the isolation of trans-product (**20**), despite rigorous purification of aldehyde **19**.

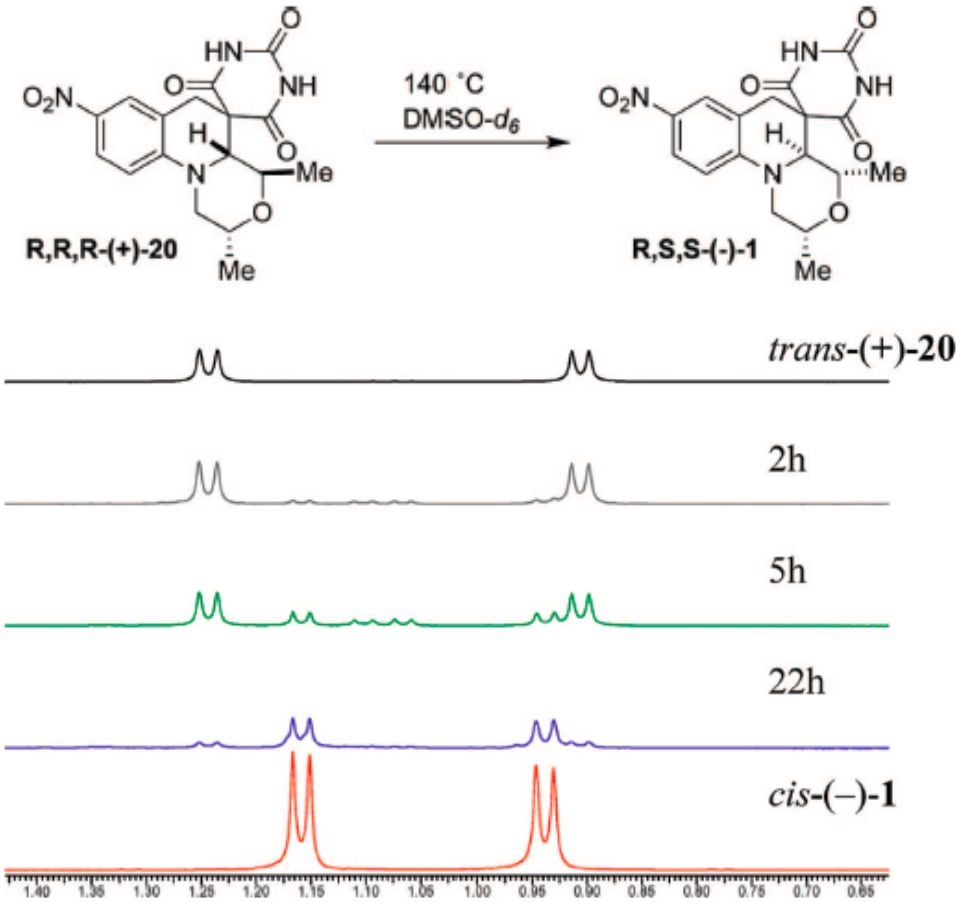
- Both the C(4) and C(4a) centers were stereochemically labile and that the configuration of the desired product may be controlled by a single persistent stereocenter at C(2).

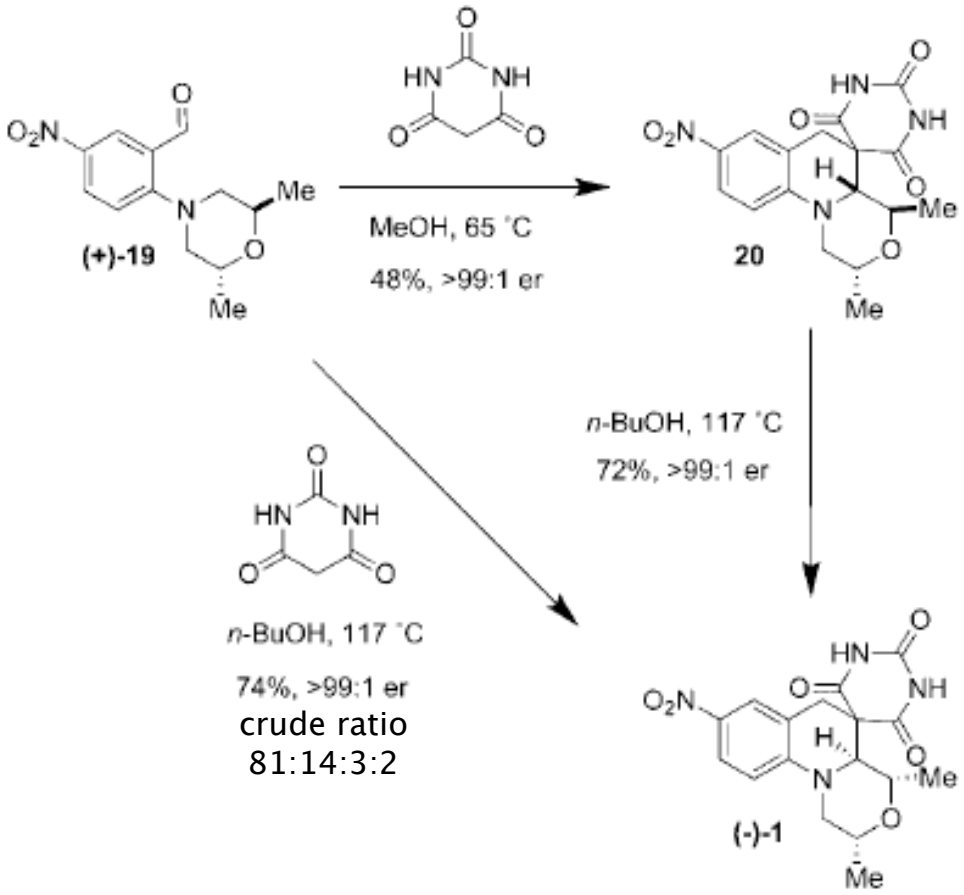
Asymmetric Synthesis of (-)-PNU-286607





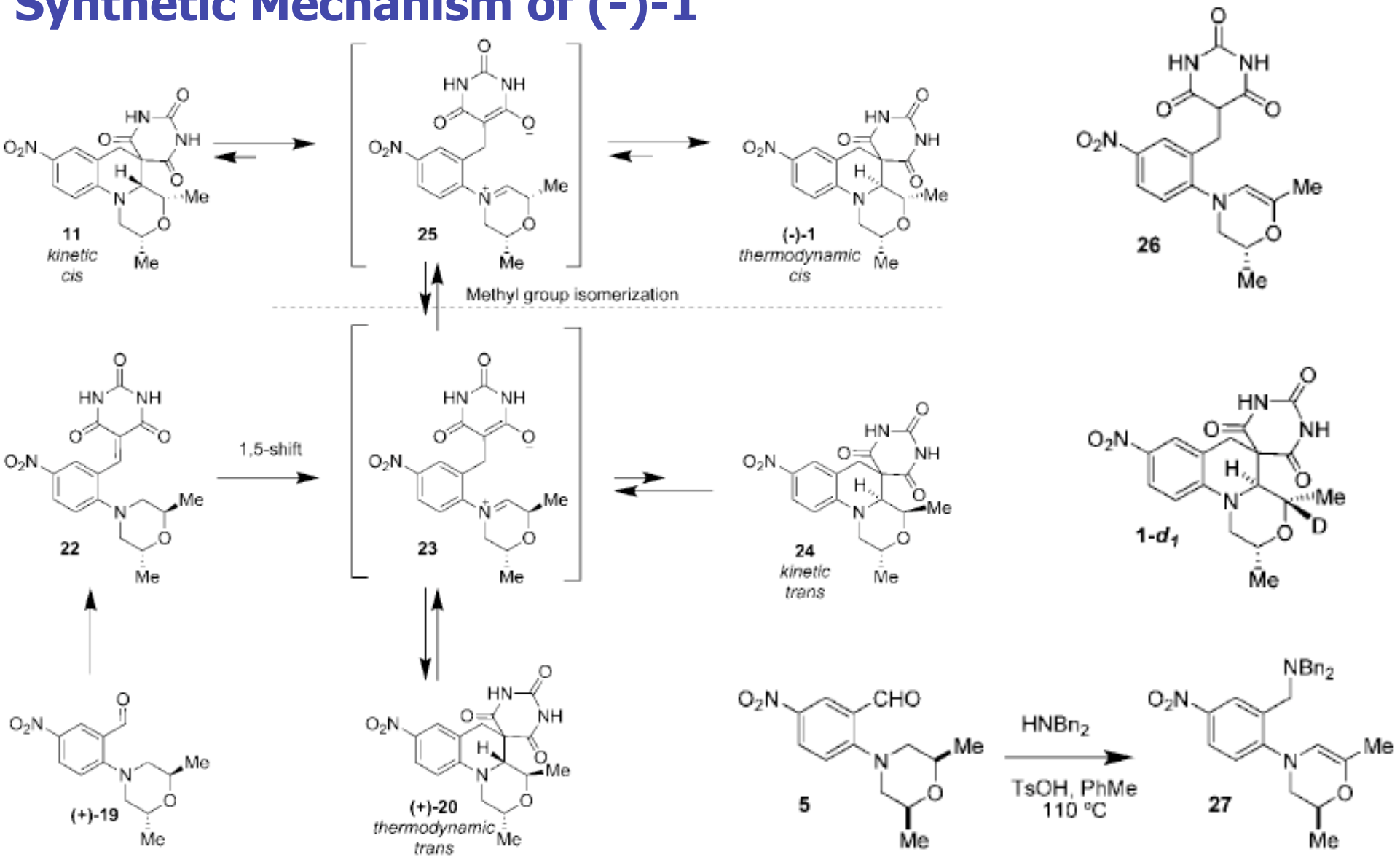
Asymmetric Synthesis of (-)-PNU-286607





- In NMR study, equilibrium was established as an ~8:1 mixture of cis/trans isomers after 22 hours.
- Practically, protic solvents were found to be optimal, providing adequate temperature control and favorable reaction rates.

Synthetic Mechanism of (-)-1



- Heating of isomerically pure (–)-**1** or (+)-**20** to 117 °C in *n*-butanol yields the identical ratio of diastereomers further indicating that the process is under thermodynamic control. However, 1,5-shift appears to be irreversible, as there is no erosion of the enantiomeric ratio of recovered (–)-**1**.