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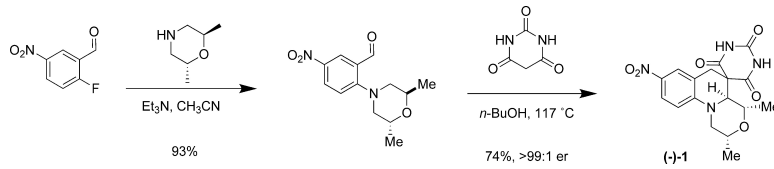

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Synthesis of (−)-PNU-286607 by Asymmetric Cyclization of Alkylidene Barbiturates

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Abstract: PNU-286607 is the first member of a promising, novel class of antibacterial agents that act by inhibiting bacterial DNA gyrase, a target of clinical significance. Importantly, PNU-286607 displays little cross-resistance with marketed antibacterial agents and is active against methicillin-resistant staphylococcus aureus (MRSA) and fluoroquinoline-resistant bacterial strains. Despite the apparent stereochemical complexity of this unique spirocyclic barbituric acid compound, the racemic core is accessible by a two-step route employing a relatively obscure rearrangement of vinyl anilines, known in the literature as the "*tert*-amino effect." After a full investigation of the stereochemical course of the racemic reaction, starting with the meso cis-dimethylmorpholine, a practical asymmetric variant of this process was developed.

Introduction

PNU-286607 was identified during a screening effort at Pharmacia and Upjohn for compounds possessing whole cell antibacterial activity.[1] A reverse chemical genomics approach led to the identification of bacterial type II topoisomerase enzymes (DNA gyrase and topoisomerase IV) as the mechanistic targets for this compound.[2] The fluoroquinolones and novobiocin have provided ample precedent that these enzymes are essential in bacteria and are appropriate antibacterial targets. Further, PNU-286607 displays little cross-resistance with marketed antibacterial therapies.[2] 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.[3,4]

Upon attempts to resynthesize the originally assigned structure of PNU-286607, it became clear that the structure was incorrect.

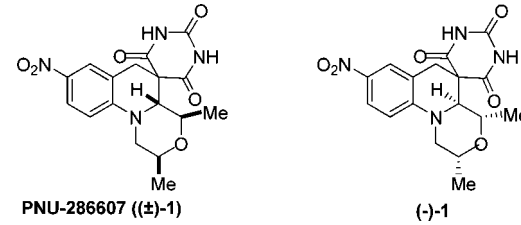

Figure 1. Structure of PNU-286607 and the active enantiomer (−)-**1** as determined by NMR analysis and X-ray crystal structure analysis.

Extensive NMR studies and an X-ray crystal structure determination revealed that PNU-286607 had the structure shown in Figure 1.[2] 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 ((−)-**1**),[2] an asymmetric synthesis was given high priority.[5]

A careful review of the literature revealed an obscure, but seemingly well-studied set of reactions influenced by what has been termed the "*tert*-amino effect." Although this reaction has been reviewed,[6] perhaps the most authoritative body of work

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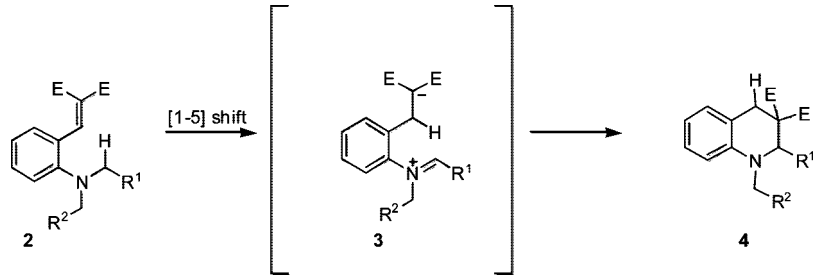
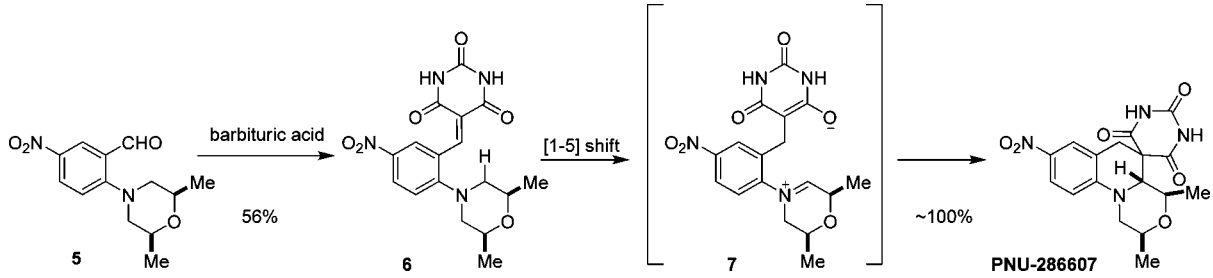
[▽] Current address: AstraZeneca Pharmaceuticals LP, 35 Gatehouse Dr., Waltham, MA 02451.
[○] Current address: Pfizer Research Technology Center, Cambridge, MA 02139.

(1) Barbachyn, M. R.; Bundy, G. L.; Dobrowolski, P. J.; Hurd, A. R.; Martin, G. E.; McNamara, D. J.; Palmer, J, R.; Romero, D. L.; Romero, A. G.; Ruble, J. C.; Sherry, D. A.; Thomasco, L. M.; Toogood, P. L. U.S. Patent 7,208,490 B2, April 24, 2007 (filed October 2, 2003).
(2) Miller, A. A. et al *Antimicrob. Agents Chemother.* **2008**, *52* (8), 2806.
(3) Spellberg, B.; Powers, J. H.; Brass, E. P.; Miller, L. G.; Edwards, J. E, Jr. *Antimicrob. Res. Dev.* **2004**, *38*, 1279.
(4) (a) Levy, S. B. *Adv. Drug Delivery Rev.* **2005**, *57*, 1446. (b) Peterson, L. R. *Clin. Microbiol. Infect.* **2005**, *11* (5), 4. (c) Silver, L. L.; Bostian, K. A. *Antimicrob. Agents Chemother.* **1993**, *37*, 377.
(5) The absolute configuration of (−)-**1** was determined by X-ray analysis of a camphanic amide of the reduced nitro derivative. See ref 2.
(6) (a) Meth-Cohen, O.; Suschitzky, H. *Adv. Heterocyl. Chem.* **1972**, *14*, 211. (b) Quintela, J. M. *Recent Res. Devel. Org. Chem.* **2003**, *7*, 259.

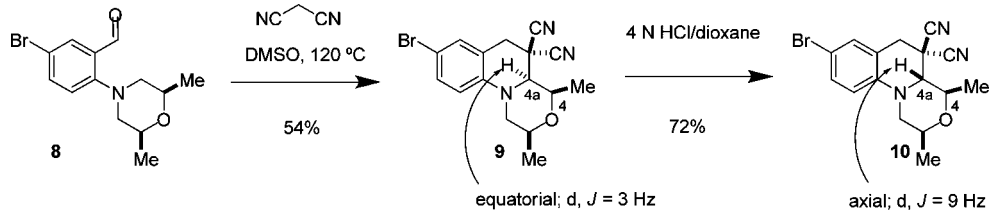
Scheme 1. *tert*-Amino Cyclizations



Scheme 2. Cyclization of Aldehyde **5** to PNU-286607



Scheme 3. Cyclization of Malononitrile Adducts of Aldehyde **8**



outlining the scope and limitations of this reaction comes from work of Verboom and Reinhoudt.[7] Additionally, some recent developments of a variant of this reaction have been reported by the Sames and Chatani laboratories.[8] The *tert*-amino effect, generically illustrated in Scheme 1, involves the 1,5-hydrogen shift of a 2-vinyl *N*-alkyl aniline (**2**) followed by cyclization of the newly generated stabilized anion on to the iminium ion of **3**, producing **4**. Typically, very strong electron withdrawing moieties are required for the initial [1,5]-shift. At the start of our investigations, there were very few published examples involving diastereoselective *tert*-amino reactions wherein the resident stereogenic centers were located on the pendant azacycle,[7d] and none with a stereogenic center located adjacent to the migrating hydrogen.[9]

Results and Discussion

Preliminary studies with meso aldehyde **5** (Scheme 2) revealed that simple mixing and heating of **5** with barbituric acid in methanol provided good yields of PNU-286607, with all the substituents around the morpholine ring in an equatorial position.[1,2,10] However, a clear understanding of the stereochemical course of the reaction was lacking

The first indication of the complexities of the reaction arose when we studied the reaction of malononitrile with aldehyde **8**, Scheme 3. Instead of the expected reaction product with the all-equatorial arrangement of substituents around the morpholine ring (**10**), the ring juncture stereochemistry was inverted; bis-nitrile **9** was the observed product. Upon stirring of **9** in 4 N HCl/dioxane for 1 h, bis-nitrile **10** was formed. The stereochemical assignments of **9** and **10**, were easily confirmed by ^{1}H NMR analysis. In particular, the coupling constants between hydrogens H(C4) and H(C4a) were diagnostic ($J = 3$ Hz for compound **9**, $J = 9$ Hz for compound **10**).

The observation of an initially formed kinetic product in the malononitrile series led us to consider whether the same pathway is operational in the barbituric acid series as well. We undertook a time course ^{1}H NMR analysis, and a deuterium labeling experiment aimed at outlining the stereochemical course of this reaction.

(7) (a) Verboom, W.; Reinhoudt, D. V.; Visser, R.; Harkema, S. *J. Org. Chem.* **1984**, *49*, 269. (b) Nijhuis, W. H. N.; Verboom, W.; Reinhoudt, D. N. *Synthesis* **1987**, *7*, 641. (c) Nijuis, W. H. N.; Verboom, W.; El-Fadl, A. A.; Harkema, S.; Reinhoudt, D. N. *J. Org. Chem.* **1989**, *54*, 199. (d) Nijhuis, W. H. N.; Verboom, W.; El-Fadl, A. A.; van Hummel, G. J.; Reinhoudt, D. N. *J. Org. Chem.* **1989**, *54*, 209. (e) Groenen, L. C.; Verboom, W.; Nuhuis, W. H. N.; Reinhoudt, D. N.; Van Hummel, G. J.; Feil, D. *Tetrahedron* **1988**, *44*, 4637.

(8) (a) Pastine, S. J.; McQuaid, K. M.; Sames, D. *J. Am. Chem. Soc.* **2005**, *127*, 12180. (b) Tobisu, M.; Chatani, N. *Angew. Chem., Int. Ed.* **2006**, *45*, 1683.

(9) After the completion of our study, a cyclization of a 3,5-dimethylpiperidine derivative was reported.; Deeva, E. V.; Glukhareva, T. V.; Zybina, N. A.; Morzherin, Y. Y. *Russ. Chem. Bull., Int. Ed.* **2005**, *54*, 1537.

(10) Our initial racemic synthesis appeared in the published patent literature (WO 2004/031195 A1, submitted on October 2, 2003 and published on April 15, 2004). Subsequently, two papers were submitted and published describing the synthesis of some compounds similar to PNU-286607: D'yachenko, E. V.; Glukhareva, T. V.; Dyudya, L. V.; Eltsov, O. V.; Morzherin, Y. Y. *Molecules* **2005**, *10*, 1101. Krasnov, K. A.; Kartsev, V. G. *Russ. J. Org. Chem.* **2005**, *41*, 901.

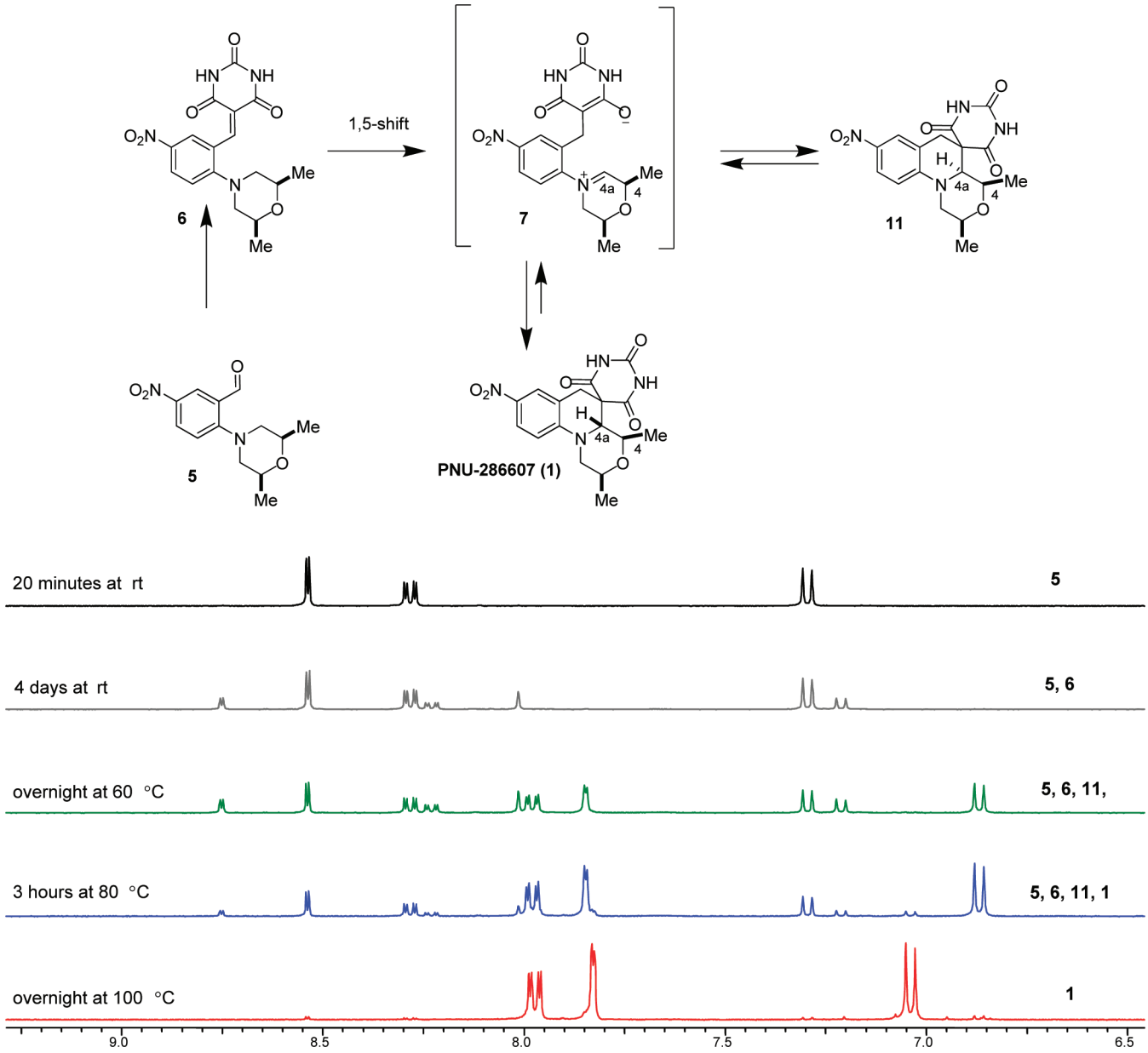
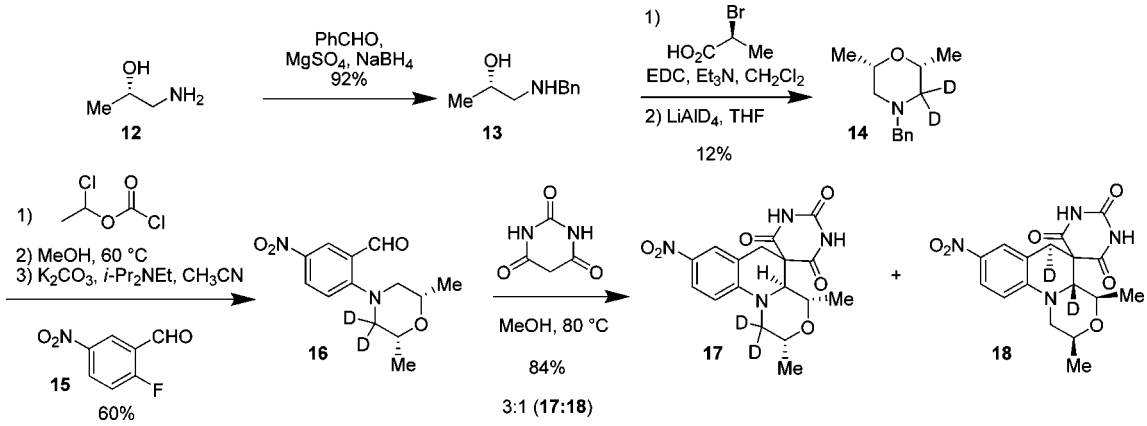


Figure 2. Time course ^{1}H NMR spectra of the conversion of **5** to PNU-286607 via **11** in DMSO-d_6 (aromatic region only).

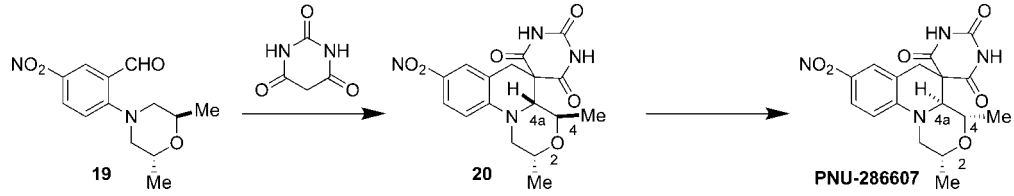
Because of the technical challenges of the experiment, namely the faster rate of the barbituric acid cyclization and unknown solubility of all the potential intermediates in this pathway, aldehyde **5** and barbituric acid were combined in DMSO-d_6 and ^{1}H NMR spectra were obtained at various time intervals and temperatures (Figure 2, only the aromatic region is shown for clarity). After 20 min at room temperature, only the starting aldehyde **5** was observed. After 4 days at room temperature an intermediate accumulated that was shown by independent synthesis to be the alkylidene intermediate **6**. Upon heating to 60 °C and then to 80 °C, a new, previously unidentified species accumulated. Further heating of the mixture at 100 °C resulted in conversion of this intermediate to the known product, PNU-286607 (**1**), which is presumably the thermodynamically more stable isomer. We speculate that the previously unidentified intermediate is the kinetically formed isomer **11**. A definitive assignment, usually accomplished by examination of the aliphatic region of the ^{1}H NMR spectra, was complicated by overlap of resonances from multiple species.

Next, we studied the [1,5]-shift using a nonsymmetrically deuterated morpholine. Reinhoudt and co-workers have investigated the [1,5]-shift with pyrrolidine-2,2,5,5-d_4.[7e] In this study the isotope effect was determined to be 3.0 ± 0.3 at 91.2 °C in DMSO-d_6, providing significant support for hydrogen migration in the rate-determining step. We were interested in studying a nonsymmetrically labeled morpholine for deeper mechanistic insight and ultimately to examine the possibility of developing an asymmetric process. First, chiral nonracemic, doubly labeled morpholine **14** was prepared by adaptation of methods previously reported by our co-workers (Scheme 4). (*S*)-1-Aminopropan-2-ol (**12**) was benzylated via reduction of the corresponding imine,[11] which was followed by an acylation−alkylation procedure with bromopropionic acid and $LiAlD_4$ reduction. During this sequence, considerable epimerization occurred,

Scheme 4. Preparation and Cyclization of Deuterium-Labeled PNU-286607



Scheme 5. Isomerization of the trans Isomer **20** to PNU-286607



resulting in the need for chromatographic separation of the disastereomers and accounting for the low yield of the desired *cis*-dimethylmorpholine. Amine deprotection followed by addition to 2-fluoro-5-nitrobenzaldehyde (**15**) furnished the cyclization precursor (**16**). To achieve a cyclization, aldehyde **16** was combined with barbituric acid and heated to 80 °C in methanol. The resulting products were isolated as a 3:1 mixture of **17** and **18**. The products were separated by CSP-HPLC and the structures were assigned by ^{1}H NMR analysis (Supporting Information). The migration of the deuterium was stereoselective in compound **18.** The ratio of product derived from a hydrogen migration (**17**) to product derived from deutero migration (**18**) was ~3:1, in line with the previously reported deuterium isotope effect.[7e] We were pleased to detect only two distinct products and not products derived from double deuterium migration, which would indicate a reversible 1,5-hydrogen migration. This synthesis of **17** represents the first asymmetric synthesis of PNU-286607, albeit in deuterated form. Further, the absolute configuration of the active enantiomer ((−)-**1**) was reconfirmed since **17** and (−)-**1** displayed equal antibacterial activity; **18** was devoid of activity.

Next, we turned our attention to the development of a synthetic route to nondeuterated asymmetric products. We had already determined that the C(4a) stereocenter (Figure 2) was stereochemically labile and was dependent upon the configuration of the adjacent methyl group at C(4) (vide supra). The doubly equatorial arrangement of the methyl groups in the 2,6-dimethyl morpholine ring was thought to be the major factor that led to the high levels of diastereoselectivity that we observed in the synthesis of PNU-286607. However, examination of the C(4a) isomerization mechanism (Figure 2) led us to consider whether the C(4) methyl-bearing stereocenter was labile as well, via the putative iminium ion intermediate **7**, Figure 2. A breakthrough observation occurred when a small amount of the cis product (PNU-286607) was observed during the isolation of *trans*-dimethylmorpholine product (**20**), derived from aldehyde **19**, Scheme 5. While this observation could have been explained by the presence of a *cis*-dimethylmorpholine contaminant in **19**, PNU-286607 was consistently observed despite rigorous purification of aldehyde **19**. *This result indicated that 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).* An enantioselective synthesis of (−)-**1** was designed around this observation. Whereas the racemic target, PNU-286607, was prepared from the meso morpholine aldehyde **5**, the synthesis of (−)-**1** was planned using chiral, nonracemic *trans*-dimethylmorpholine intermediate (+)-**19**, Figure 3.

Capitalizing on the observed isomerization, to develop a robust, scalable, and practical procedure was anticipated to be a considerable challenge. It was unclear whether the energetic differences between the two compounds (**20** and PNU-286607, Scheme 5) were sufficient to drive the equilibration to synthetic

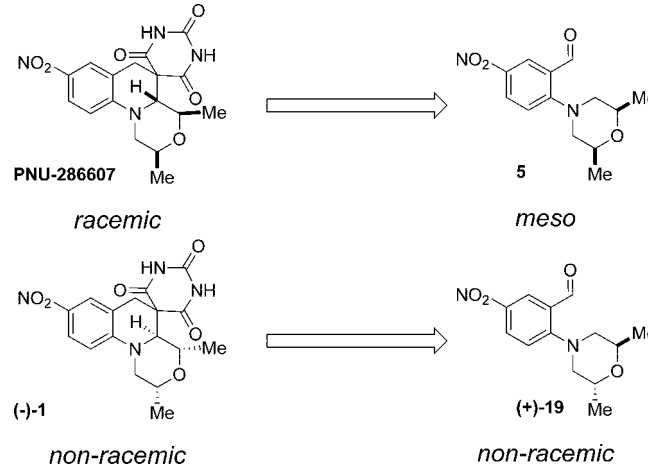


Figure 3. Synthetic design for the asymmetric synthesis of (−)-**1**.

(11) A similar procedure has been reported for the synthesis of the enantiomer of the aminoalcohol: Mickelson, J. W.; Belonga, K. L.; Jacobsen, E. J. *J. Org. Chem.* **1995**, *60*, 4177.

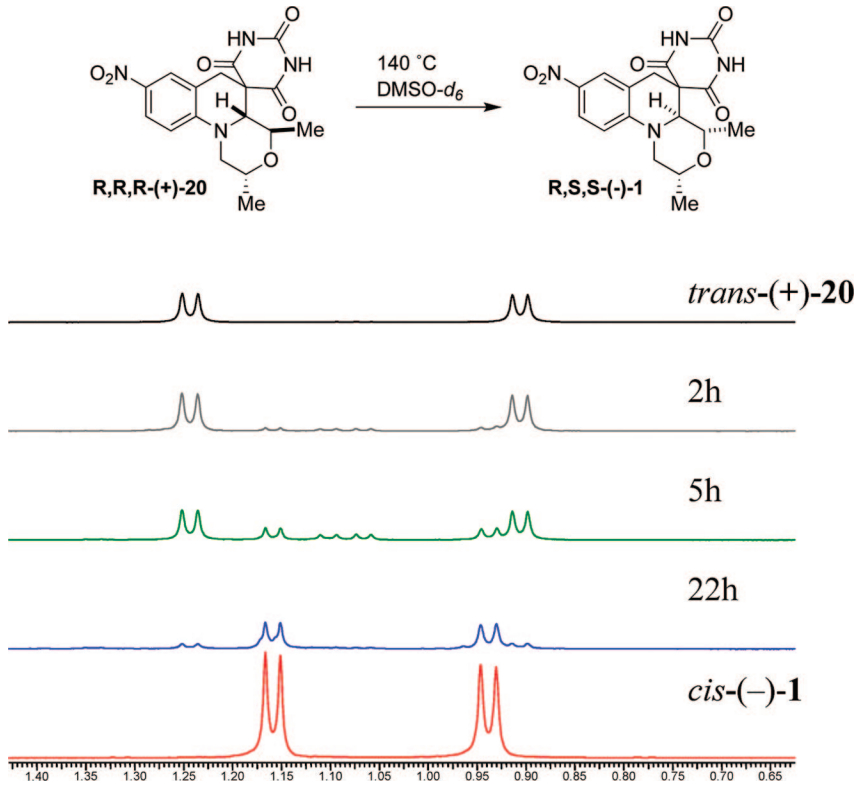
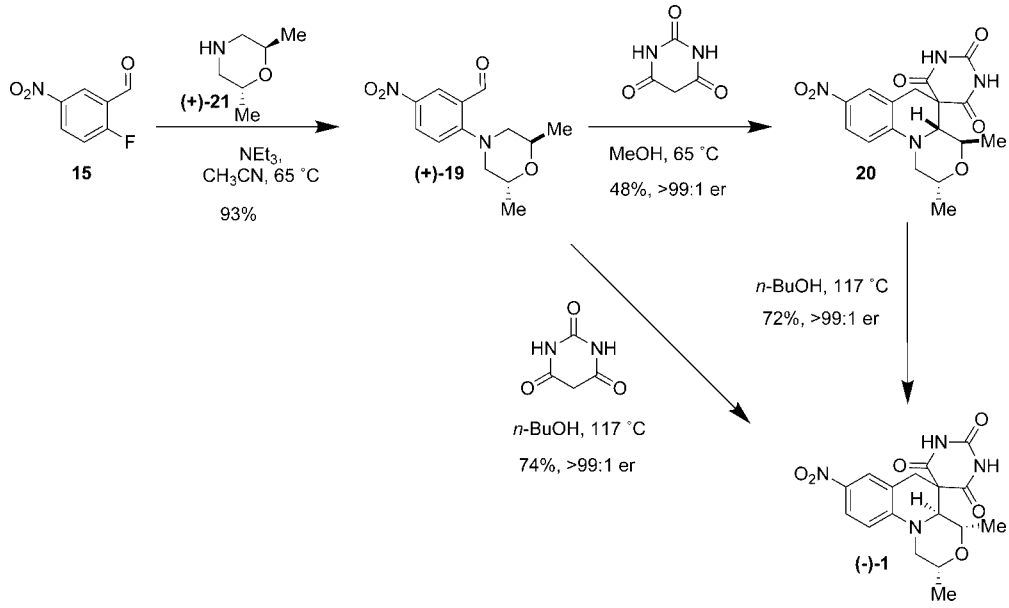


Figure 4. ^{1}H NMR spectra (methyl region) of the isomerization of (+)-**20** to (−)-**1** in DMSO-d_6.

Scheme 6. Asymmetric Synthesis of (−)-**1**, the Active Enantiomer of PNU-286607



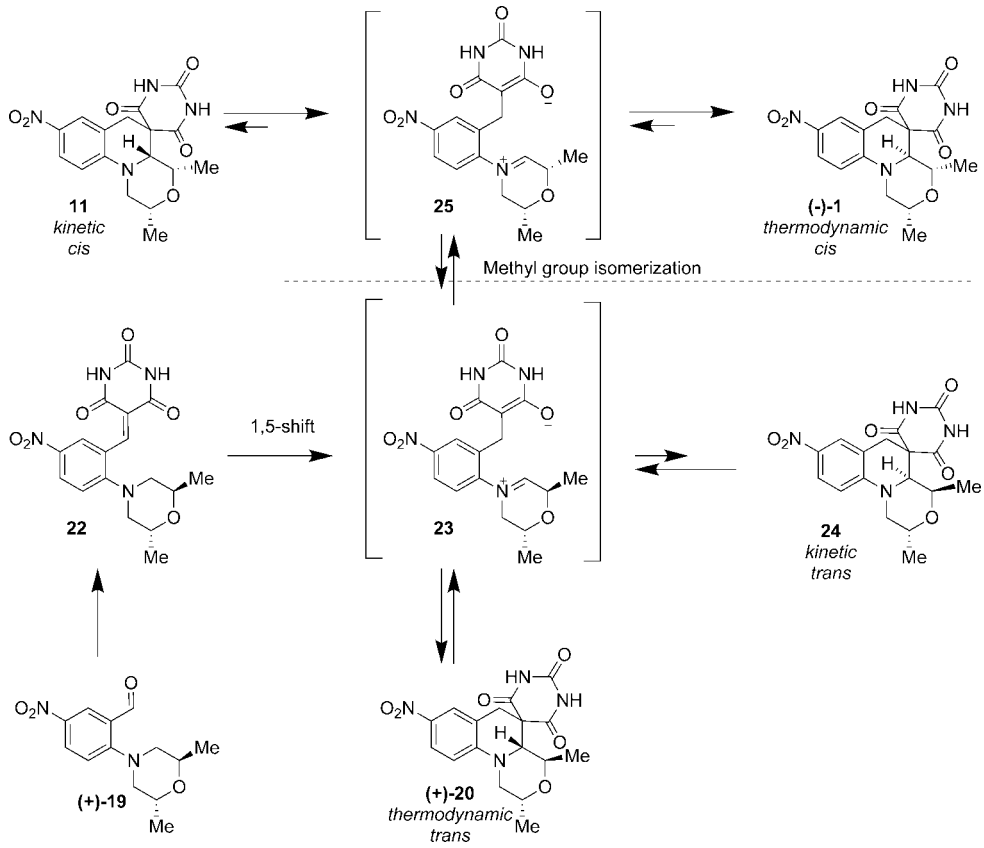
cally acceptable levels. Further, we anticipated that the experimental conditions necessary to achieve the isomerization might be quite vigorous and we were concerned about the durability of the products under these conditions.

We began our investigation into the asymmetric synthesis by first monitoring the isomerization using ^{1}H NMR spectroscopy. Figure 4 shows the methyl region of the ^{1}H NMR time course in DMSO-d_6 for the isomerization of (+)-**20** to (−)-**1**. After 2 h at 140 °C, two sets of new resonances appeared which became more abundant at 5 h. By 22 h, equilibrium was established as an ~8:1 mixture of cis/trans isomers. As a proof of concept, this ratio was deemed to be acceptable.

Practically, protic solvents were found to be optimal, providing adequate temperature control and favorable reaction rates. Aldehyde (+)-**19**, prepared by the addition of chiral morpholine **21**[12] to aldehyde **15,** was treated with barbituric acid and MeOH and heated to 65 °C (eq 1, Scheme 6). This effected the conversion of (+)-**19** to trans product (+)-**20** in 48% yield. The trans product **20** then was isomerized in 72% yield to (−)-**1** by

(12) (2*R*,6*R*)-2,6-Dimethylmorpholine was purchased from BASF.

Scheme 7. Mechanism of [1,5]-Shift, Cyclization Producing (+)-**20** and Isomerization to (−)-**1**



simple heating in *n*-butanol at 117 °C. Further, the process was reduced to a one-step procedure by dissolution of aldehyde (+)-**19** and barbituric acid in *n*-butanol and heating to reflux for 24 h (Scheme 6). The ratio of crude products was 81:14:3:2, but the desired product (−)-**1** was isolated in 74% yield and >99:1 er, providing a two-step asymmetric synthesis of (−)-**1**, the active enantiomer of PNU-286607.

Mechanism. The proposed mechanism of the [1,5]-hydride shift, cyclization sequence in the meso series to the *kinetic* product **11** is illustrated in Figure 2. The first step, formation of the alkylidene (**6**) occurs readily at room temperature and is usually accompanied by a color change from the bright yellow of the aldehyde to the deep red color of the alkylidene. Alkylidene **6** has been isolated and characterized, and is competent in the thermal conversion to PNU-286607. The deuterium-labeling experiment lends support to the intramolecular, stereoselective hydride migration being the key step, although the experiment fails to indicate which hydride (deuterium) migrates, axial or equatorial, because of epimerization at the newly formed methine bridgehead position (C4a). However, it is reasonable to conclude that the axial hydride migrates due to preferential orbital overlap with the coplanar vinyl aryl system. The product of kinetic control **11** arises by axial attack of the enolate on the iminium ion. In the subsequent isomerization, **11** thermally reverts via a retro-Mannich type process to zwitterion **7** followed by closure to PNU-286607, either via a higher energy transition state structure with *equatorial* attack of the enolate on the imminium ion, or via *axial* attack on a higher energy morpholine conformation. Either mechanism gains access to the more thermodynamically stable product with the all-equatorial arrangement of substituents around the morpholine ring.

Extension of this mechanistic analysis to the isomeric morpholine (+)-**19** is illustrated in the lower portion of Scheme 7. Alkylidene **22**, derived from barbituric acid condensation with aldehyde (+)-**19**, undergoes the [1,5]-shift producing zwitterion **23**. Presumably, the product of kinetic control **24** arises via axial attack of the enolate on the iminium ion. Although product **24** was never identified and characterized, there were four isomers produced in the asymmetric cyclization. The least abundant isomer is presumed to be **24**, the *kinetically* formed trans product.[13] Reversion to zwitterion **23** and subsequent cyclization occurs via a higher energy transition state structure with equatorial attack, or alternatively via a higher energy morpholine ring conformation to provide (+)-**20**, the *thermodynamically* more stable trans isomer.

When sufficient energy is applied to the system, zwitterion **23** isomerizes to zwitterion **25**, giving access to the cis manifold. Attack of the enolate produces either the cis product from kinetic control (**11**), or the cis product from thermodynamic control (−)-**1**. Because of the all-equatorial arrangement of the substituents around the morpholine ring, (−)-**1** is the lowest energy and most abundant of the four isomers formed. In the above stereochemical analysis, the *kinetic* versus *thermodynamic* designation refers to the relationship between the stereogenic centers at C(4) and C(4a) and the cis and trans designation refers to the relationship of the two methyl groups across the morpholine system (Figure 2 and Scheme 7). 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, the [1,5]-

(13) Attempts to characterize **24** were hampered by its low abundance and poor stability when isolated in enriched form.

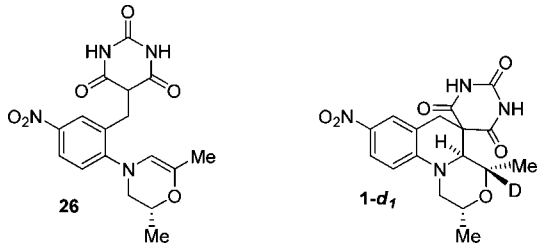
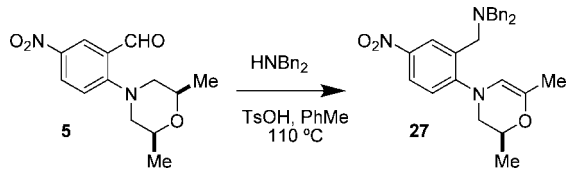


Figure 5. Putative enamine intermediate **26** and deuterium incorporation product **1-*d*$_1$**.

Scheme 8



shift appears to be irreversible, as there is no erosion of the enantiomeric ratio of recovered (−)-**1**.

Presumably, the isomerization of zwitterion **23** to **25** proceeds via the enamine **26**, Figure 5. Although enamine **26** could not be detected, we present two pieces of evidence that support the intermediacy of **26**. First, heating aldehyde **5** with dibenzylamine and *p*-toluenesulfonic acid produces enamine **27,** itself an interesting product derived from the [1,5]-shift of a hydride onto the dibenzyliminium ion, Scheme 8. Product **27** lends support for the intermediacy of the morpholino-enamine substructure **26**. Second, when the [1,5]-shift, cyclization, isomerization sequence (Scheme 5) was conducted in *n*-butanol-d_1, deuterium incorporation occurs at the C4 position affording **1-*d*$_1$** (Figure 5), providing further support for the intermediacy of enamine **26** in the isomerization process.[14]

Conclusion

We have developed a practical two-step asymmetric synthesis of the (−)-enantiomer of PNU-286607, a promising member of a novel class of antibacterial agents currently under development. We have highlighted mechanistic aspects of the reaction that permit stereochemical control over the reaction course and the isolation of products of high enantiomeric purity. Our mechanistic understanding of the *tert*-amino effect as it applies to PNU-286607 allowed for the use of chiral *trans*-dimethylmorpholine as the source of asymmetry to obtain a product containing an embedded *cis*-dimethylmorpholine. Although only a single methyl stereocenter around the morpholine ring persists during the process, the trans arrangement is necessary for introduction of chirality.

Acknowledgment. The authors thank Gary Martin for extensive NMR analysis of PNU-286607, **9**, and **10** and Fusen Han for the X-ray crystal structure of PNU-286607. We also acknowledge Jeremy Starr for assistance with analytical data.

Supporting Information Available: General experimental details; full experimental details including spectroscopic and analytical data for compounds (−)-**1**, **8**−**10, 13, 14, 16**−**20**, and **27**; X-ray crystal structure of PNU-286607. This material is available free of charge via the Internet at http://pubs.acs.org.

JA808014H

(14) Additionally, a significant literature precedent exists for a similar methyl group isomerization in Sapogenin to iso-Sapogenin. Woodward, R. B.; Sondheimer, F *J. Am. Chem. Soc.* **1958**, *40*, 6693.