ORGANIC LETTERS

2012
Vol. 14, No. 14
3632–3635

Access to the Pactamycin Core via an Epoxide Opening Cascade

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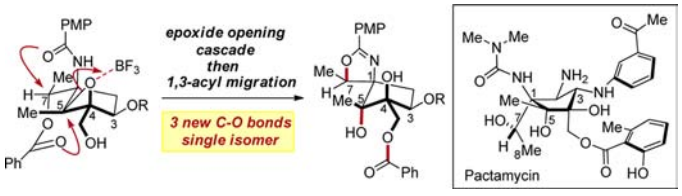
Received May 28, 2012

ABSTRACT



A synthetic strategy to establish five contiguous stereocenters, in a stereocontrolled manner, on the core structure of pactamycin is described. This sequence exploits the use of a Lewis acid mediated epoxide opening cascade to set the relative configuration of the C4−C5 diol while reversing the configuration at C7. This sequence provides the oxygenated core of pactamycin in just 11 steps.

Isolated from the fermentation broth of *Streptomyces pactum* var. *pactum*,[1] pactamycin (**1**) is a highly potent antiproliferative agent, displaying activity against all three phylogenetic domains (eukarya, bacteria, and archaea). When tested against eukaryotic cell lines pactamycin generally displays nanomolar cytotoxicity (KB, IC_{50} = 5.3 nM; MCR-5, IC_{50} = 53 nM; HCT116 $IC_{50} \approx$ 100 nM).[2,3] Pactamycin is active against both gram-negative and gram-positive bacteria[4] and has antiproliferative effects against the protozoan parasites *Plasmodium falciparum* (IC_{50} = 14 nm) and *Trypanosoma brucei* (IC_{50} = 7 nm).[2]

Pactamycin's activity stems from its ability to inhibit protein synthesis.[5,6] Given its broad-spectrum activity, it was hypothesized that it must interact with a conserved region of the ribosome. Originally thought to bind the P-site,[7,8] it was later shown that it most likely binds the highly conserved E site of the 30S ribosomal subunit.[9] This was further supported by the identification of functional mutants of *H. halobium* resistant to pactamycin.[10] It was ultimately shown, with atomic resolution, that pactamycin does bind a single site on the 30S ribosome.[11] In doing so, it distorts the position of the mRNA such that it cannot interact with a neighboring E-site bound tRNA. This prevents the initiation of protein synthesis ultimately leading to cell death.

Small structural variations, as seen in the related natural products 7-deoxypactamycin (**2**)[12] and jogyamycin (**3**)[13] (Figure 1), suggest that functional group changes can have dramatic effects on both potency and selectivity in regard to antiproliferative effects between kingdoms.[13] Recent work by Mahmud et al. has shown that biosynthetic

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10.1021/ol301461e
Published on Web 07/03/2012

manipulations can generate alternate analogues of **1**,[3] more specifically TM-025 (**4**) and TM-026 (**5**) which display activity against both chloroquine resistant and chloroquine sensitive *P. falciparum* (IC_{50} = 25−30 nm), but display significantly reduced cytotoxicity toward mammalian cells (HCT116, IC_{50} > 1 mM). While it is unclear if these analogues maintain pactamycin's mechanism of action, it does suggest that this scaffold holds promise for the development of efficacious agents against biomedically significant pathogens.

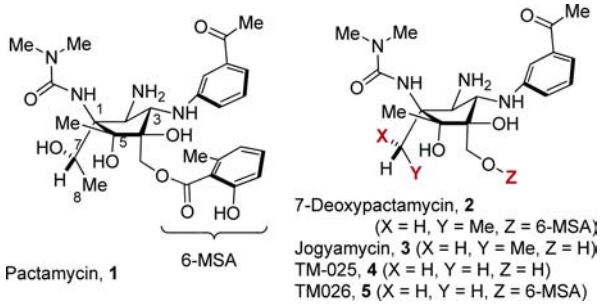

Figure 1. Pactamycin and its structural relatives.

Pactamycin's structure, elucidated in 1972, is highlighted by six contiguous stereogenic centers, three of which are quaternary, with all five positions of the cyclopentane core functionalized.[14] Being the most complex of the aminocyclopentitol antibiotics, it is not surprising that the chemical synthesis of pactamycin presents a significant challenge. Only two approaches to **1** had been reported prior to the recent total synthesis by Hanessian's group.[15,16] Even with this monumental accomplishment, structural manipulations to the scaffold are hampered by its extreme complexity, and its synthetic difficulty has prevented any noteworthy structure−activity relationship studies.

Concurrently with Hanessian's efforts, we were interested in designing a modular synthesis of pactamycin to address these issues. Similar in approach, we had recognized threonine as an obvious synthon from which to begin our campaign as it contains the stereochemcial information needed at C7 and C1. However, a key feature in our approach was to relay the stereochemistry at C7 to the antidiol functionality present at C4/C5. We envisioned that an intramolecular epoxide opening by a pendant carbonate group on O7′ might serve well to establish the relative stereochemistry at C4−C5 as depicted in Figure 2A.[17] A hypothetical transition state leading to the ring-opened epoxide, however, reveals a highly destabilizing *syn*-pentane interaction if the correct stereochemistry at C7 is present.

This led us to consider a ring-opening cascade in which the incorrect stereochemistry at C7 would be introduced but inverted by a cascade cyclization of a pendant amide on N1 (Figure 2B). Herein we report our progress in implementing this strategy.

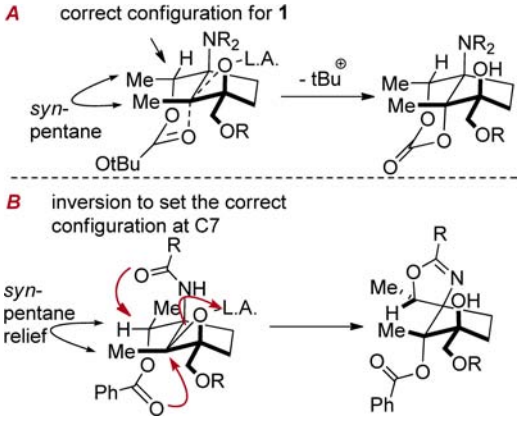

Figure 2. O7-assisted epoxide ring-opening cascade design.

Installation of the opposite stereochemistry at C7 is easily accomplished by formation of the oxazoline (**6**) from L-threonine as described by Balavoine.[18] The enolate of **6** can be alkylated with 5-iodo-1-pentene to give **7** (Scheme 1).[19] Significant optimization was required to inhibit β-elimination of the enolate, identifying HMPA as a key additive. Under optimized conditions, **7** could be obtained as a single diastereomer in 80% yield.[20] This reaction is noteworthy in that it can be done on a 100 g scale requiring only a water workup to remove HMPA and no chromatography. The methyl ester was then converted to the methyl ketone **8** in a single operation utilizing conditions reported by Grabowski and co-workers.[21] The terminal olefin was then oxidatively cleaved to give the aldehyde **8**. An intramolecular aldol condensation promoted by SiO_2 and Hünig's base in toluene at 70 °C afforded the spirocyclic cyclopentenal **9** in 75% yield. Oxidation of the unfunctionalized cyclopentane carbon at C3 was accomplished by radical bromination.[22] To our delight this provided **10** as a single diastereomer, albeit in modest yield. As long as reaction

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(15) For partial syntheses, see: (a) Knapp, S.; Younong, Y. *Org. Lett.* **2007**, *9*, 1359. (b) Tsujimoto, T.; Nishikawa, T.; Urabe, T.; Isobe, M. *Synlett*. **2005**, 433. (c) Malinowski, J. T.; McCarver, S. J.; Johnson, J. S. *Org. Lett*. **2012**, *14*, 2878.

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(17) For selected epoxide cascade reactions, see: (a) Vilotijevic, I.; Jamison, T. F. *Angew. Chem., Int. Ed.* **2009**, *48*, 5250. (b) Feng, X.; Shu, L.; Shi, Y. *J. Am. Chem. Soc.* **1999**, *121*, 11002. (c) Molander, G. A.; Pozo Losada, C. D. *J. Org. Chem.* **1997**, *62*, 2935. (d) Minami, A.; Migita, A.; Inada, D.; Hotta, K.; Watanabe, K.; Oguri, H.; Oikawa, H. *Org. Lett*. **2011**, *13*, 1638.

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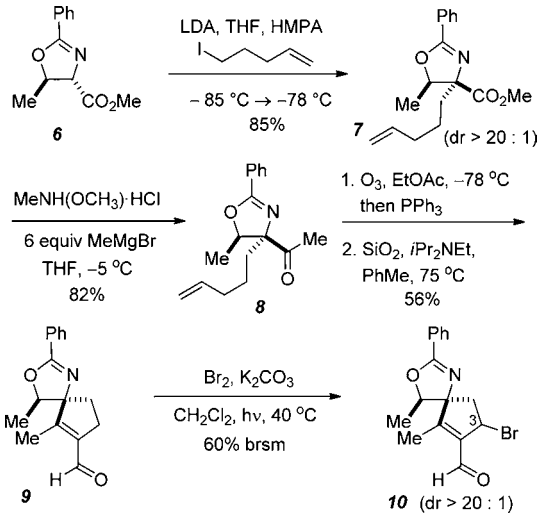
times were kept short, a significant amount of starting material could later be reisolated and recycled. Longer reaction times led to substantial amounts of decomposition.

Scheme 1. Synthesis of the Key Spirocyclic Cyclopentenal

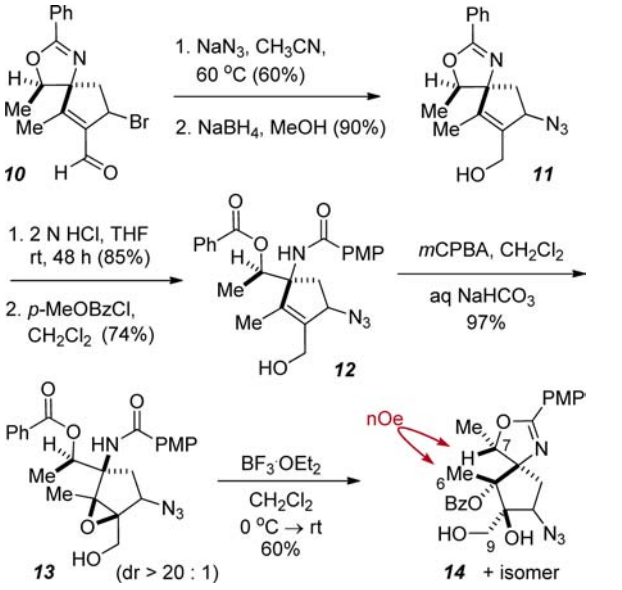


While the allylic bromide **10** was produced as a single diastereomer, the relative stereochemistry at C3 could not be unambiguously assigned. Alternative reactions on substrates similar to **9** (e.g., epoxidation of the C4–C5 alkene) had demonstrated that addition to the spirocyclic cyclopentene occurs from the same face as N1.[23] This is presumably a consequence of the C7 methyl group being positioned directly under the cyclopentene. This led us to believe that the bromide might produce the correct relative stereochemistry at C3 after substitution with a nitrogen based nucleophile.

Attempts to reduce the aldehyde in **10** with borohydride reagents in alcoholic solutions led to either alcoholysis or reduction of the highly reactive allylic bromide. The bromide could be cleanly displaced, however, with sodium azide first, and then the aldehyde cleanly reduced to provide **11** (Scheme 2).

With the azido alcohol in hand, hydrolysis of the oxazoline afforded the free primary amine at C1 which was reacylated with *p*-MeOBzCl to give **12**. Epoxidation of the alkene with *m*-CPBA produced **13** in good yield and as a single diastereomer. Again, epoxidation was presumed to have occurred from the top face as shown, due to the C7-substituents shielding the bottom face of the cyclopentene core. Screening a variety of Lewis acids identified BF_3•OEt_2 as an active catalyst to trigger the cascade rearrangement to provide **14**. NOE studies on **14** suggested that the rearrangement had proceeded with inversion at C7 as evidenced by a strong correlation between the proton on C7 and those on C6. Further structural studies were hampered by the presence of a structural isomer of **14** which we presumed to be a product arising from migration of the benzoyl group. It was hypothesized that 1,3-acyl migration had occurred to the primary alcohol at C9 as evidenced by significant deshielding of the AB quartet signal from the C9 methylene protons in the ^{1}H NMR spectrum.

Scheme 2. Key Cascade Sequence with Azide **11**



To simplify our investigations we attempted a global hydrolysis of the benzoate esters which might be present. Treatment of **14** with LiOH afforded a single compound (**15**), confirming that these were indeed structural isomers (Figure 3). Compound **15** was a crystalline solid, with which we were able to confirm the structure by X-ray crystallography.[24] This confirmed that we had successfully inverted the stereochemistry at C7 and had established the correct relative stereochemistry of the C4–C5 diol. However, this revealed that the stereochemistry of the azide at C3 was incorrect.

With the improper stereochemistry at C3 we sought an alternative strategy that would permit double inversion of this center to access the correct diastereomer. To accomplish this we first treated **10** with sodium acetate under solvolysis conditions.

This cleanly delivered the C3-alcohols **16** and **17** as an ~1:1 mixture of diastereomers (Scheme 3); presumably the intermediate acetates are cleaved during the reaction. Analysis of the ^{1}H NMR spectrum of the diastereomer **16** revealed an identical ^3J splitting pattern as the azide **15** (dd, ^3J = 6.0, 6.0 Hz) suggesting that it carried the inverted center at C3. The other diastereomer **17** lacks coupling to one of the methylene protons at C2 (d, ^3J = 7.5 Hz) consistent with the bromide **10**, indicating it retained the original stereochemistry at C3.

Able to identify both diastereomers, we endeavored to stereoselectively introduce an oxygen nucleophile at C3. To our surprise, substitution with sodium benzoate produced

(23) See "An explanation of oxazoline directed stereocontrol" in the Supporting Information for details.

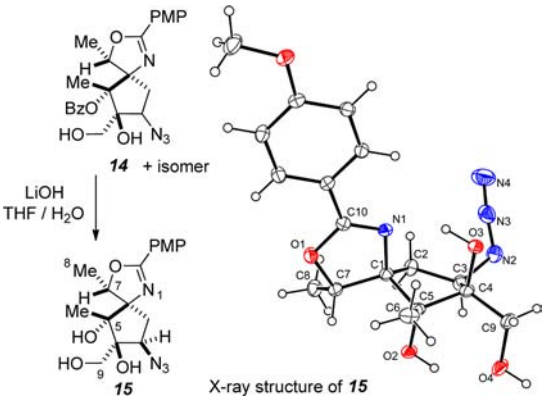
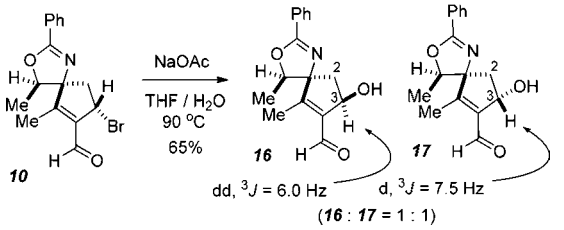


Figure 3. Confirmation of the stereochemistry at C3.

Scheme 3. Solvolysis of the Allylic Bromide

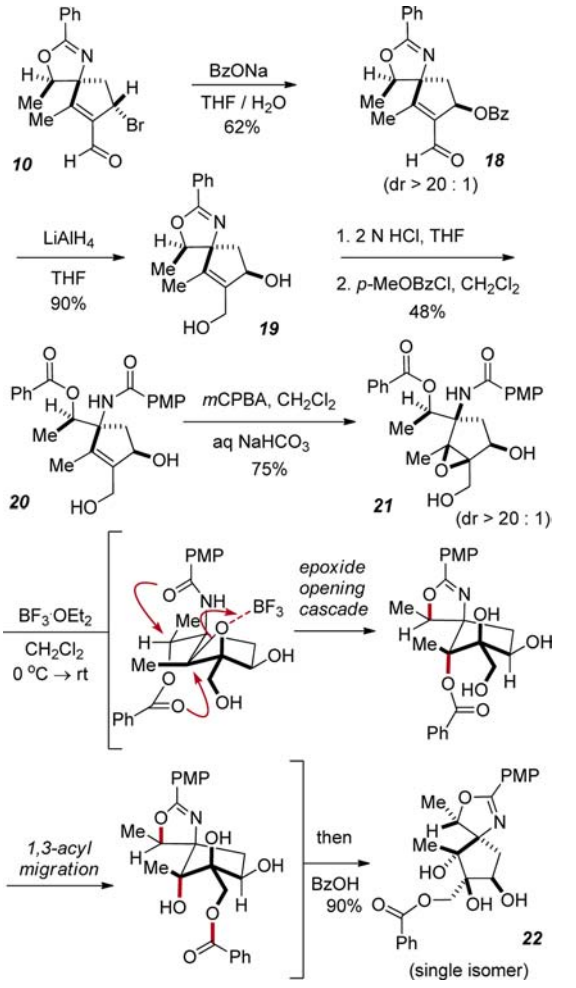


18 as a single diastereomer consistent with complete inversion, and with the benzoate intact (Scheme 4). Following introduction of the benzoate, simultaneous reduction of the aldehyde and the C3 ester with $LiAlH_4$ provided the diol **19**. Hydrolysis of the oxazoline and reacylation of the N1 amine provided **20**. As before, epoxidation of the alkene gave **21** as a single diastereomer. Treatment of **21** with $BF_3{\bullet}OEt_2$ successfully triggered the cascade reaction, to again give a mixture of benzoate isomers. It was found that protic acids were effective at completing the 1,3-acyl migration. Thus, treatment of the reaction mixture with excess benzoic acid gave **22** as a single isomer in 90% yield.

In summary, an efficient 11-step sequence to access the oxygenated pactamycin core is described. This approach is highlighted by an epoxide opening cascade which ultimately delivers five of the six contiguous stereocenters in a stereocontrolled manner. Studies to install the C2–C3 antidiamine functionality from the C3 alcohol are currently underway. The brevity of this approach should

(24) This structure has been deposited in the Cambridge Crystallographic Data Centre (CCDC No. 887952).

Scheme 4. Key Cascade Sequence and 1,3-Acyl Migration



strengthen our ability to identify functional groups critical to achieving selectivity between organisms with this complex antibiotic scaffold.

Acknowledgment. We are grateful to the University of Utah for financial support. We thank Dr. Atta Arif (University of Utah) for help in determining the structure of **15** and Dr. Jim Muller (University of Utah) for assistance with mass spectral analyses.

Supporting Information Available. Experimental and characterization details, including ^{1}H and ^{13}C NMR spectra for new compounds **6**–**10** and **16**–**22**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.