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Asymmetric Synthesis and Biological Activities of Pactamycin-Inspired Aminocyclopentitols

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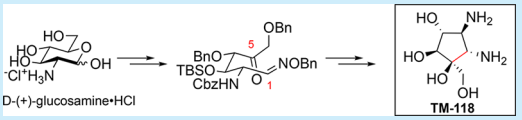
Supporting Information

ABSTRACT: Pactamycin is a structurally unique aminocyclitol antibiotic with broad-spectrum cell growth inhibitory activity. To explore the bountiful activity of the aminocyclitol core of pactamycin, an efficient, modular, and asymmetric synthesis of aminocyclopentitols resembling the pactamycin pharmacophore has been developed employing a SmI_2-mediated imino-pinacol coupling strategy. Two of the compounds exhibited antitumor activity against A375 melanoma cells.



Natural products continue to play a vital role in drug discovery. Approximately two-thirds of small molecule pharmaceuticals currently on the market are natural products or derived from natural product structures.[1] Among the plethora of bioactive natural products is pactamycin (Figure 1), a potent antitumor antibiotic produced by the soil bacterium *Streptomyces pactum*.[2] Pactamycin is a member of the aminocyclopentitol family of microbial secondary metabolites whose structures commonly derive from carbohydrates.[3] Aminocyclopentitols cover a broad swathe of biological activity, some of which include glycosidase inhibitors and antitumor, antimicrobial and antiviral agents (Figure 1).[4] Unfortunately, the biological activity displayed by pactamycin spans across all three phylogenetic domains,[2,5−7] where its indiscriminate cytotoxicity toward mammalian cells has suppressed its development toward therapeutic application. Nevertheless, the pactamycin pharmacophore is believed to be a wellspring of promising biological activity that is waiting to be harnessed. For example, we have demonstrated, through biosynthetic manipulations, production of new pactamycin analogs that are more active against malarial parasites, *Plasmodium falciparum*, than toward bacteria and mammalian cells.[8−11] We have subsequently elucidated the tailoring steps in pactamycin biosynthesis[11,12] and obtained intermediate compounds that also showed potent biological activities. Continuing our efforts to draw further on the bountiful activity of the aminocyclitol core of pactamycin, we have taken a synthetic approach to access the aminocyclopentitol ring, which could open up a diverse library of biologically active compounds.

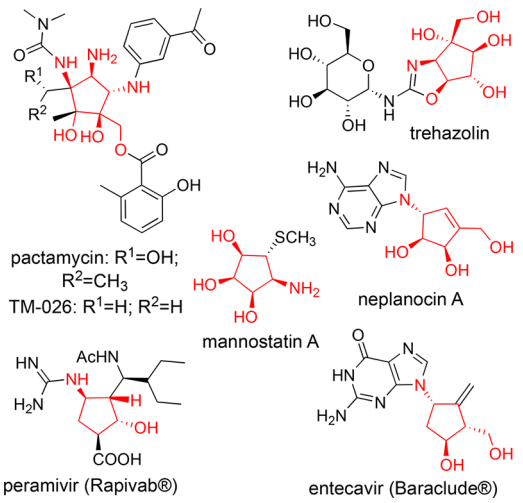
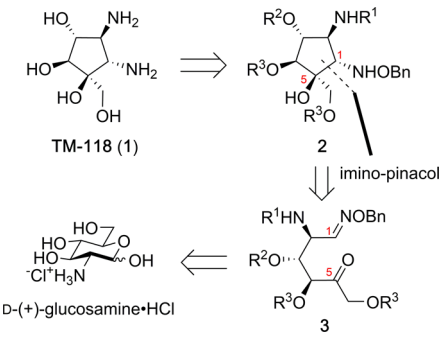


Figure 1. Chemical structures of bioactive natural products and clinically used drugs that contain aminocyclopentitol units (red).

Scheme 1. Retrosynthesis of TM-118



The intricate aminocyclopentitol structure of pactamycin, harboring 6-contiguous stereocenters, has garnered considerable attention from the synthesis community[13−19] over the years, which only more recently culminated in the landmark total synthesis by Hanessian in 2011,[20] followed by the elegant and efficient total synthesis of Johnson in 2013.[21]

The structurally complex aminocyclitol core is expected to be the source of the bioactivity of pactamycin and its congeners. As such, we set out to synthesize the aminocyclopentitol core lacking the aromatic rings, methyl groups, and the *N*,*N*-dimethyl

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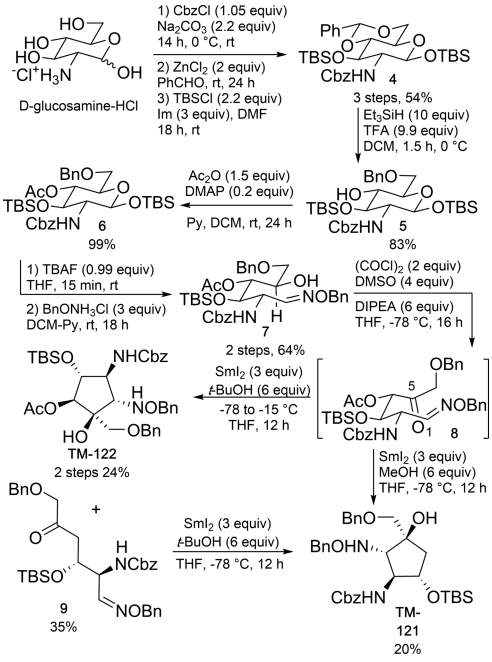
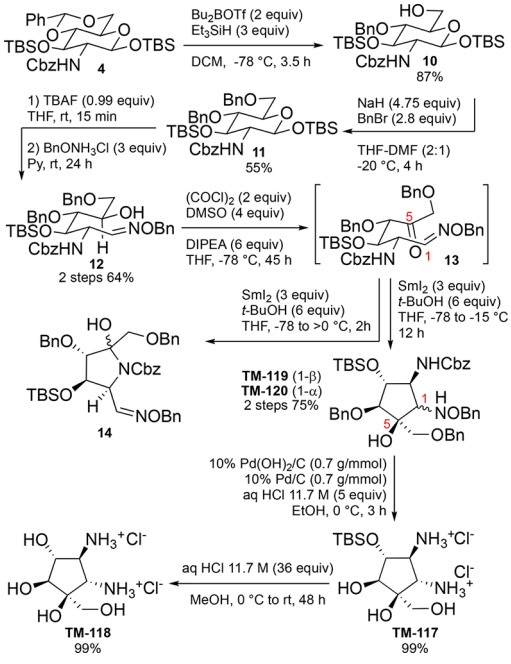
Scheme 2. Synthesis of Aminocyclopentitols



Scheme 3. Synthesis of TM-117−TM-120



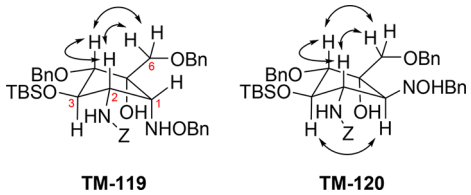


Figure 2. Key NOE correlations of the imino-pinacol products **TM-119** and **TM-120**.

urea, all of which have been shown to affect the cytotoxicity of pactamycin or its analogs to some extent.[7,9,11,22,23] The overarching goal was to provide modular access to a structurally

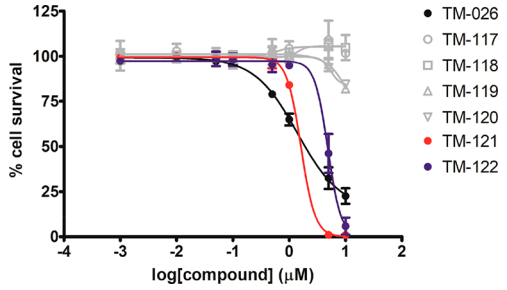


Figure 3. Growth inhibitory assay of **TM-117−TM-122** against melanoma cells *in vitro*. Dose response curves indicate A375 melanoma cells are sensitive to **TM-121**, **TM-122**, and the previously characterized analog of pactamycin (**TM-026**). All other synthetic analogs exhibited no inhibitory activity at the tested doses. Data represent mean ± SD with $N = 2$.

unique aminocyclopentitol, that itself or a direct precursor could be derivatized to suit further SAR studies.

Our retrosynthetic analysis to obtain the desired amino-cyclopentitol **TM-118** (**1**) began with deprotection of **2** (Scheme 1). An orthogonally protected diamine **2** would allow further functionalization/derivatization at nearly any position, imparting a high degree of synthetic flexibility to support future SAR studies. Diamine **2** could be constructed in a key imino-pinacol cyclization step from a 1,5-keto-aldoxime ether **3**.[24,25] The keto-oxime ether would come from a suitably protected carbohydrate derived from D-(+)-glucosamine. Aside from the cost benefit, glucosamine offers multiple advantages, e.g., it contains most of the desired functionality and 3 out of 5 of the necessary stereocenters in the product, and the stereochemistry at C-2 and C-4 could be critical for obtaining the desired diastereoselection in the key imino-pinacol coupling step.[26−29]

The forward synthesis (Scheme 2) commenced with Cbz-protection of the glucosamine nitrogen under aqueous conditions, followed by benzylidene acetalization of the 4,6-hydroxy groups, then bis-silylation of the remaining anomeric and the C-3 hydroxyls with 2 equiv of TBSCl/imidazole providing the bis-TBS benzylidene acetal **4** as a single diastereomer in 54% yield over three steps. After considerable experimentation and screening a number of known methods, we did not find suitable conditions to affect the reductive benzylidene ring opening to expose the free C-6 hydroxyl.[30,31] Instead, we found Et_3SiH/TFA to be a high yielding reagent combination for this substrate that selectively exposed the C4-OH (**5**, Scheme 2). Although we later developed new conditions that exclusively provided the exposed C6-hydroxyl intermediate **10** (Scheme 3), our first attempts at synthesizing the amino-cyclopentitol ring proceeded through the 4-OH intermediate (**5**, Scheme 2).

Initial efforts to access a protected aminocyclopentitol **2** by reductive benzylidene ring opening of acetal **4** with Et_3SiH/TFA gave the 4-OH glucopyranose intermediate **5** in good yield (Scheme 2). However, the C4 hydroxyl group on the pyranose ring proved particularly unreactive as a nucleophile. We found benzylation ineffective under a variety of conditions, and even *O*-acylation with benzoyl chloride and stoichiometric quantities of DMAP was challenging with this substrate.[31] However, acetylation gave the 4-OAc compound in high yield. While this protecting group could be problematic during the imino-pinacol cyclization, due to potential elimination of the alpha acetoxy group, we decided to carry the 4-OAc substrate forward to the imino-pinacol coupling stage for several reasons. First, in

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addition to the mild radical promoter SmI_2, there are other reagents capable of generating ketyl radicals and effective at promoting pinacol coupling reactions that could be attempted.[24,32−37] Second, we reasoned if deoxygenation were to occur, the linear deoxy-byproduct might undergo cyclization and produce a structurally unique monodeoxy-aminocyclitol product that would be an interesting substrate for later SAR studies.

A regioselective desilylation of **6** exposed the anomeric hydroxyl group in high yield providing both alpha and beta diastereomers, which could be allowed to equilibrate to the alpha diastereomer, then subjected to dehydrative ring opening to form the 5-hydroxyaldoxime **7** in moderate, but useful yield over two steps. Oxidation to the 5-keto-1-aldoxime ether would provide the imino-pinacol precursor. This was achieved using a modified Swern oxidation [$(COCl)_2$, DMSO, THF, then DIPEA, −78 to −20 °C] to give the desired 5-ketoaldoxime **8**.[38] As mentioned above, the instability of **8** could limit the reagent system that could be employed for the imino-pinacol cyclization to those capable of forming the requisite ketyl radical at low temperatures such as SmI_2 or Et_3B/O_2. Gratifyingly, a one-pot Swern oxidation/SmI_2-mediated reductive cyclization produced the desired aminocyclopentitol **TM-122**, albeit in low yield (two steps, 14%), and the linear *des*-acetoxy ketone **9** (35% yield) (Scheme 2). We observed the *N*-cyclized compound was formed when the 5-ketoaldoxime **8** from the Swern oxidation step was warmed to room temperature. While the pyrrolidine byproduct could be suppressed by keeping the Swern oxidation below −15 °C, after the addition of DIPEA, this resulted in a modest increase in yield to 24%, formation of the linear des-acetoxy **9** product could not. However, **9** was isolated and resubjected to the reductive cyclization conditions, which provided the deoxy-aminocyclopentitol **TM-121**. Interestingly, we also found that the *des*-acetoxy-aminocyclopentitol **TM-121** could be obtained directly from the Swern oxidation/SmI_2 reductive coupling sequence simply by switching the proton donor from *t*-BuOH to MeOH.[39]

The liberated C-6 alcohol **10** was eventually obtained through a regioselective reductive benzylidene ring opening (Scheme 3). By switching TFA-Et_3SiH in our first route (Scheme 2) to a Lewis acid system, Bu_2BOTf-Et_3SiH, we were able to reverse the benzylidene ring opening selectivity and obtained the desired, free C-6 alcohol **10** in high yield (87%). To our knowledge, this reagent combination is new for this type of transformation.[30,31]

Benzylation of the C-6 hydroxyl provided benzylated intermediate **11** in modest yield (55%), but suffered from competitive *N*-benzylation of the 6-*O*-benzyl product with the starting material. *N*-Benzylation could be suppressed completely by keeping the reaction below −20 °C and quenching at 50% conversion. Selective removal of the anomeric-TBS group afforded the corresponding free lactol. Literature methods for this transformation use AcOH-TBAF to buffer the basicity of the fluoride anion, but we found the reaction sluggish, requiring over 48 h to reach completion.[40] By omitting AcOH, the reaction was complete within 10 min, selective, and high yielding (90%). Dehydrative ring opening of the lactol, provided both *E*/*Z*-isomers of the 5-hydroxy-aldoxime ether **12** in 64% yield. After screening a number of oxidation conditions to obtain the 1,5-keto-aldoxime as the imino-pinacol precursor, we settled on modified Swern conditions ($COCl_2$, DMSO, DIPEA, −78 °C) but observed that the product ketone **13** was unstable above 0 °C.[38] As the reductive keto-aldoxime cyclization was expected to occur at low temperatures, we therefore attempted to telescope the Swern and imino-pinacol coupling steps to overcome the instability issue of the intermediate ketone.[26] To our delight, the Swern/imino-pinacol coupling successfully produced the orthogonally protected aminocyclopentitols **TM-119** and **TM-120** (Figure 2) as a mixture of two out of four possible diastereomers (75% yield). We have successfully repeated this two-step conversion on gram scale. Notably, Chiara and co-workers attempted similar imino-pinacol couplings with protected 2-amino-carbamate 1-aldoxime ethers but reportedly suffered from *N*-cyclization and a mixture of many products.[41] In our hands we found the *N*-cyclized compound **14** was the major decomposition product from the ketone starting material if allowed to warm above 0 °C. Importantly, this finding grants convenient access to differentially protected cyclopentyl diamines utilizing an imino-pinacol coupling strategy. While formation of the desired stereoisomer **TM-120** can be rationalized by the stereoelectronic effects noted in Figure S1, the formation of diastereomer **TM-119** is less straightforward. Interestingly, a similar erosion of stereoinduction was reported in an imino-pinacol coupling with an *O*-acetate vicinal to the radical accepting aldoxime.[42] Their reported result with the *O*-Ac was an anomaly in a series of otherwise completely diastereoselective imino-pinacol couplings. Carbonyl oxygens are known to be capable of directing SmI_2-mediated reactions.[43] We hypothesize that coordination of an organosamarium with the NH-Cbz group is likely responsible for overriding the otherwise inherent stereoelectronic bias imposed by allylic strain in conformation C of Figure S1 and producing **TM-119**.

At this stage in the synthesis, the protected aminocyclopentitol **TM-120** was used as a branching point for derivatization/SAR studies, but for our immediate purposes we desired the free aminocyclopentitol **TM-118**. However, a number of debenzylation conditions failed to deliver any desired product even with extended reaction times and higher temperatures (Table S1). Interestingly, the one electron reductant, lithium 4,4′-di*tert*-butylbiphenylide (LiDBB), furnished the putative desired intermediate **TM-117** but left the highly water-soluble aminocyclitol as an intractable mixture of mostly inorganic impurities after aqueous workup.[31] A number of nonaqueous workup/purification conditions also failed to provide **TM-117**. It is noteworthy, however, that LiDBB has not been previously demonstrated to cleave the N−O bond in hydroxylamines. Finally, using a 1:1 mixture of $Pd(OH)_2/C$ and Pd/C, which was reported to possess superior hydrogenation reactivity than either catalyst alone,[44] we successfully debenzylated the aminocyclopentitol at 0 °C (H_2, EtOH, conc. HCl, 5.2 equiv) in less than 3 h to provide the desired **TM-117** as the sole product (99% yield). Encouraged by this result, we hoped to debenzylate and remove the TBS group in one pot by switching from EtOH to MeOH as the hydrogenation solvent, but this was unsuccessful.[45] However, we observed that after full conversion to **TM-117**, followed by a filtration, solvent exchange to MeOH, and additional HCl (11.7 M, 36 equiv), full desilylation had occurred providing the desired final product **TM-118** in nearly quantitative yield (99%).

Preliminary biological testing of **TM-117**−**TM-122** against A375 human melanoma cells revealed that the deoxy-aminocyclopentitol **TM-121** and the acetate **TM-122** in a dose-dependent manner inhibited growth of cancer cells and exhibited antitumor activity, on par with the activity observed with the pactamycin derivative **TM-026** (Figure 3). The IC_{50} values (95% CI) for **TM-026**, **TM-121**, and **TM-122** are 1.405 (0.9474 to 2.084) μM, 1.619 (1.393 to 1.881) μM, and 4.861 (3.300 to 7.161) μM, respectively. The lack of activity of **TM-118** suggests

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that additional side chains to the core aminocyclitol are necessary for the compounds to show appreciable cell inhibitory activity.

In conclusion, we have developed efficient asymmetric routes to the aminocyclopentitol **TM-118** and its analogs. Further, we have demonstrated that some of our pactamycin-inspired synthetic compounds have appreciable biological activity and display promising antitumor activity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b03681.

Experimental procedures, $[\alpha]_D$, IR, NMR, and MS data for compounds **4**−**7**, **9**−**12**, and **TM-117**−**TM-122**, and their 1D and 2D NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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