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Natural Product Synthesis

Total Synthesis of Pactamycin**

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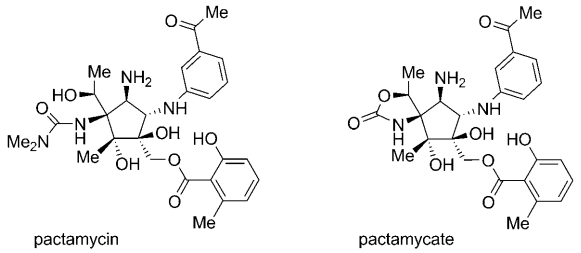
Among the plethora of microbial secondary metabolites produced by the soil bacterium of the *Streptomyces* family is pactamycin, a structurally unique member of aminocyclopentitol-containing natural products (Scheme 1).



Scheme 1. Structures of pactamycin and pactamycate.

Pactamycin was isolated in 1961 from a fermentation broth of *Streptomyces pactum* var *pactum* by scientists at the former Upjohn Company.[1] It exhibits activity against Gram-positive and Gram-negative bacteria, in addition to potent in vitro and in vivo cytotoxic effects.[2] Its further development as a chemotherapeutic agent was curtailed owing to its toxicity. The potent protein synthesis inhibitory activity of pactamycin is attributed to the stage of translocation from the A and P sites to the P and E sites during formation of certain *m*-RNA-*t*-RNA complexes in prokaryotes as well as in eukaryotes.[3] Pioneering X-ray crystallographic studies[4] involving binding to the 30S site of *Thermus thermophilus* show unique interactions, whereby pactamycin adopts a spatial orientation so as to mimic an RNA nucleotide. The two aromatic moieties stack against each other like consecutive RNA bases, while the core cyclopentane motif mimics the RNA sugar-phosphate backbone, which results in an intricate network of hydrogen-bonded interactions within the 30S site of the ribosome. Recent elegant studies on the biosynthesis of pactamycin by Mahmud and coworkers[5a] revealed a gene cluster which also produced pactamycate, de-6-MSA-pactamycin and de-6-MSA-pactamycate, the natural congeners lacking the 6-methyl salicylic acid moiety.[5b–d]

A proposed structure of pactamycin was reported in 1970 by the Upjohn scientists as a result of seminal studies involving chemical degradation.[6] It was subsequently corrected in 1972 as a result of X-ray crystallographic studies, as shown in Scheme 1.[7] To the best of our knowledge, pactamycin is the most densely functionalized naturally occuring aminocyclopentitol.[8] In spite of its unique architecture and rich history in the realm of RNA structure and function,[3–5] efforts toward the synthesis of pactamycin and its congeners have been sparse. Knapp and Yu,[9] as well as Isobe and coworkers[10] recently reported conceptually different approaches toward the construction of the aminocyclopentane core motif. Herein, we communicate the first total synthesis of pactamycin and its naturally occurring congener, pactamycate (Scheme 1).

In considering a synthetic strategy, we were cognizant that the densely functionalized cyclopentane core harboring three contiguous tertiary centers would require a judicious choice of well-orchestrated bond-forming sequences. Furthermore, we wanted to adopt a modular approach for the introduction of substituents and appendages to allow for diversification to eventually prepare bioactive analogues while eliminating toxicity.

Analysis of the structure of pactamycin led to the choice of L-threonine as a partially hidden chiron, representing C1, C2, C7, and C8, and ensuring the configuration of the secondary hydroxy group in the hydroxyethyl appendage, as well as the position of the amine group at C1 (Scheme 2). The cyclopentenone core (**C**) would arise from a sequence of well-documented reactions culminating with an intramolecular aldol condensation. Systematic manipulation of the cyclopentenone (**C**) would then eventually lead to pactamycin. Straightforward as this plan may have been, its execution was met with several unexpected roadblocks particularly involving the elaboration of the *N*,*N*-dimethylurea group at C1, and the proximity of functional groups (see below).

A three-step sequence starting with L-threonine (**1**) led to the oxazoline derivative **2** (Scheme 3).[11,12] Formation of the enolate with LiHMDS, and condensation with O-TBDPS-2-hydroxymethyl acrolein, and subsequent protection with TESOTf afforded **3** as a single isomer. Reduction of the benzyl ester to the aldehyde, treatment with MeMgBr, and oxidation afforded the methyl ketone **4**. Ozonolytic cleavage of the exocyclic methylene group, followed by a highly stereoselective Mukaiyama-type intramolecular aldol condensation afforded **5** as a crystalline intermediate.[13] Upon treatment with trichloroacetyl chloride in pyridine, β-elimination took place to give the cyclopentenone **6**.

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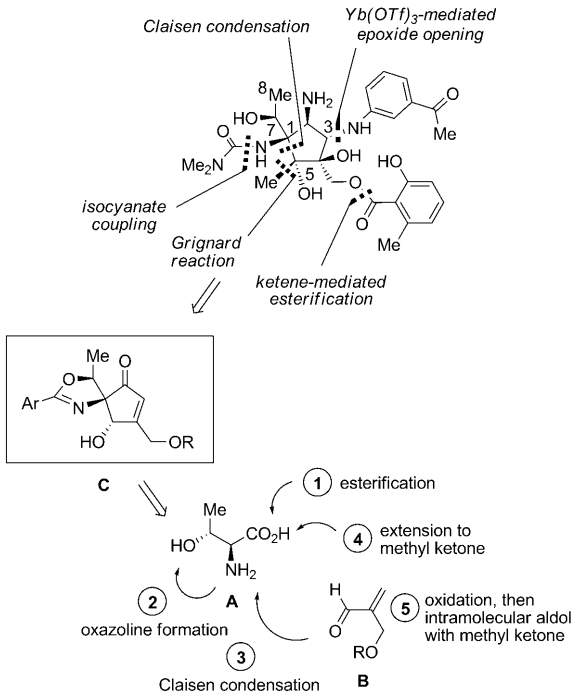
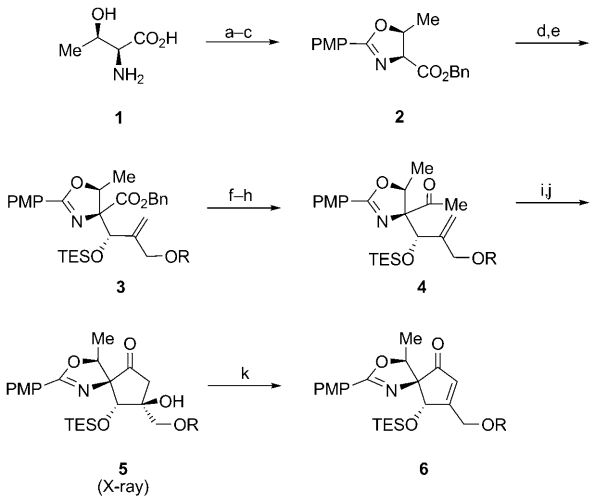
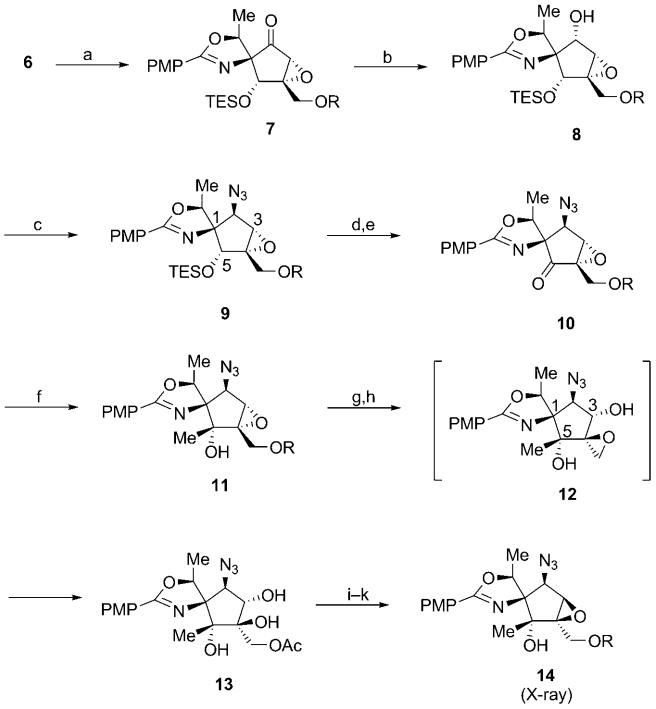


Scheme 2. Strategic bond disconnections and key transformations shown in their order of execution. **A** = L-threonine, **B** = oxygen-protected 2-hydroxymethyl acrolein, **C** = core cyclopentenone intermediate.



Scheme 3. Synthesis of the cyclopentenone intermediate **6**. Reagents and conditions: a) BnOH, PTSA, benzene, reflux, 67%; b) *p*-anisoyl chloride, TEA, CH_2Cl_2, RT, 64%; c) $SOCl_2$, MeCN, 0°C, 85%; d) LiHMDS, THF, −78°C, O-TBDPS-2-hydroxymethylacrolein; e) TESOTf, 2,6-lutidine, CH_2Cl_2, 0°C to RT, 67% (over 2 steps); f) DIBAL-H, CH_2Cl_2, −78°C; g) MeMgBr, Et_2O, 0°C, 87% (over 2 steps); h) $(COCl)_2$, DMSO, TEA, CH_2Cl_2, −78°C to RT, 91%; i) O_3, CH_2Cl_2, −78°C, then DMS, 84%; j) $TiCl_4$, CH_2Cl_2, DIPEA, TMSCl, 0°C, 85%; k) Cl_3CCOCl, py, CH_2Cl_2, RT, 89%. Bn = benzyl, DIBAL-H = diisobutylaluminum hydride, DIPEA = *N*,*N*-diisopropylethylamine, DMS = dimethyl sulfide, DMSO = dimethyl sulfoxide, HMDS = 1,1,1,3,3,3-hexamethyldisilazane, PMP = *p*-methoxyphenyl, PTSA = toluene-*p*-sulfonic acid, py = pyridine, R = TBDPS = *tert*-butyldiphenylsilyl, TEA = triethylamine, TES = triethylsilyl, Tf = trifluoromethanesulfonyl, THF = tetrahydrofuran.

From this point on, it was imperative to introduce the epoxide and hydroxy group in that order, while securing the desired configurations that would allow introduction of an azide group with inversion at C2, and the aniline moiety regioselectively at C3. In the event, base-induced epoxidation of **6** afforded epoxide **7**, which was stereoselectively reduced under Luche conditions to give **8** (Scheme 4). The formation



Scheme 4. Synthesis of the epoxide **14**. Reagents and conditions: a) H_2O_2 (30% w/w), 20% NaOH, MeOH/CH_2Cl_2 (7:1), 0°C, 75%, (88% bsmr); b) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH/CH_2Cl_2 (1:1), 0°C, 92%; c) Tf_2O, py, −78°C to 0°C, then Bu_4NN_3, toluene, RT, 87%; d) TFA/MeCN/H_2O (1:8:1), 0°C to RT, 93%; e) DMP, CH_2Cl_2, 0°C, 96%; f) MeMgBr, THF, −78°C; g) TBAF, THF, 0°C, 86% (over 2 steps); h) $Zn(OTf)_2$, AcOH, 80°C; i) K_2CO_3, MeOH, RT; j) TBDPSCl, TEA, DMAP, RT, 85% (over 3 steps); k) Tf_2O, py, CH_2Cl_2, −78°C to 0°C, 96%. DMAP = 4-dimethylaminopyridine, DMP = Dess–Martin periodinane, R = TBDPS, TBAF = tetra-*n*-butylammonium fluoride, TFA = trifluoroacetic acid.

of the α-oriented epoxide and secondary alcohol as in **8** was imperative, because the S_N2 azide displacement of the corresponding triflate in the diastereomeric β-epoxide in a related series was unsuccessful. Although the azide group could be easily introduced through the triflate ester of **8** to give **9**, it became necessary to "invert" the configuration of the epoxide to contemplate the stereo- and regioselective introduction of the aniline moiety by opening at C3. This operation was postponed in favor of the obligatory carbon-methyl branching at C5. Thus, selective cleavage of the TES ether and oxidation to the ketone gave **10**, which was treated with MeMgBr to give **11** as a single diastereomer. An X-ray structure of the phenyl oxazoline analogue of **10** confirmed its structure and absolute configuration.[12,13] The "inversion" of

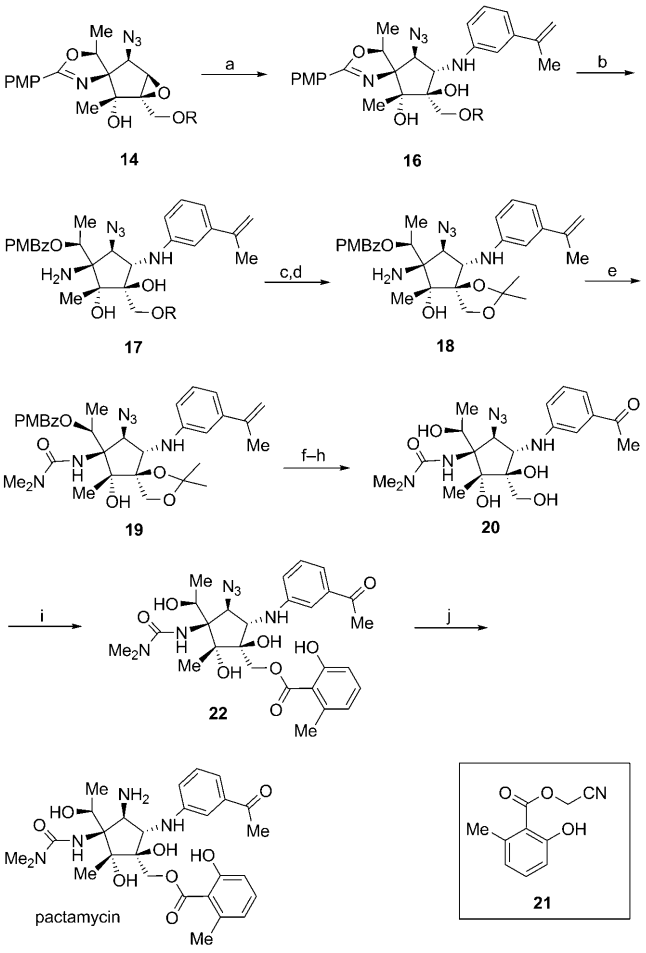
the epoxide in **11** was achieved by treatment of the corresponding primary alcohol with $Zn(OTf)_2$ in AcOH to give the triol **13** with C4 inversion. Presumably, this arose from the spiroepoxide **12** which underwent solvolysis to afford the primary acetate as in **13**. A two-step sequence restored the robust TBDPS ether group, and the resulting triol was converted in situ into the epoxide **14** through the secondary triflate (70% overall yield from **11**). An X-ray crystal structure validated the suggested sequence of inversions in going from **11** to **14**.[13]

Highly stereoselective epoxide opening at C3 with the aniline derivative **15** in the presence of $Yb(OTf)_3$,[14] afforded the core structure **16** as the sole regioisomer (Scheme 5). Cleavage of the oxazoline moiety with aqueous HCl[15] led to the *p*-methoxybenzoyl ester, which was transformed into the acetonide **18** in straightforward manner.

Formation of an intermediate isocyanate in the presence of diphosgene,[16] then treatment with dimethylamine gave the urea **19** in excellent yield. Treatment of the ester with DIBAL-H, subsequent oxidative cleavage of the olefin to the methyl ketone, and hydrolysis of the acetonide function led to **20**. Esterification with the cyanomethyl ester **21**,[17] then reduction of the azide group in the presence of Lindlar's catalyst, afforded pactamycin which was purified by chromatography on silica gel. Synthetic pactamycin exhibited spectroscopic and chiroptical properties identical to the originally published data.[6,12] Furthermore, ^{1}H NMR data at 700 MHz, ^{13}C NMR data at 100 MHz, HPLC data, as well as single crystal X-ray structures of several intermediates provide hitherto unavailable characterization features for future synthetic endeavors.[12] Pactamycin is reported to be unstable in solution as evidenced by a change in optical rotation in different solvents, thus losing some of its biological activity with time.[6]

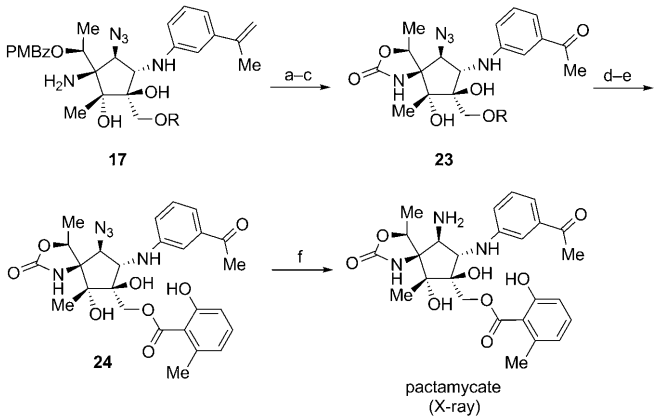
The synthesis of crystalline pactamycate,[6] a naturally occurring congener,[5a] is shown in Scheme 6. Treatment of **17** with DIBAL-H, then diphosgene,[16] resulted in the formation of the corresponding cyclic carbamate. Oxidative cleavage of the *exo*-methylene group in the latter afforded **23**, which was converted in two steps into the 2-azido precursor **24**. Hydrogenation in presence of Lindlar's catalyst gave crystalline pactamycate. An X-ray crystal structure confirmed the structure of pactamycin and the original assignment of its absolute configuration for the first time (Scheme 6).[7,12,13]

The successful and seemingly straightforward total synthesis of pactamycin described here underscores the importance (and frustrations) of many unwanted transformations caused by the proximity of reactive functional groups anchored on the cyclopentane core. For example, having introduced the primary amino group at C1 in intermediate **17** by acidic hydrolysis of the oxazoline **16**, there only remained to convert it into the *N*,*N*-dimethylurea, bringing us within a few steps from the intended target, pactamycin. In practice, various attempts at reaction of **17** with *N*,*N*-dimethylcarbamoyl chloride resulted in the formation of the precursor oxazoline **16** (Scheme 7 A). In fact, triethylamine and DMAP alone effected the same transformation into **16**. Attempted formation of the desired *N*,*N*-dimethylurea by treatment of **17** with diphosgene to give an intermediate isocyanate, then quenching with dimethylamine, formed the six-membered cyclic carbamate **25** in 91% yield even at −46 °C (Scheme 7 B)![13] This remarkably facile reaction, spanning a tertiary alcohol and a highly hindered amine, demonstrates the importance and unexpected consequences of spatial proximity in a confined architecture such as that found in **17**.

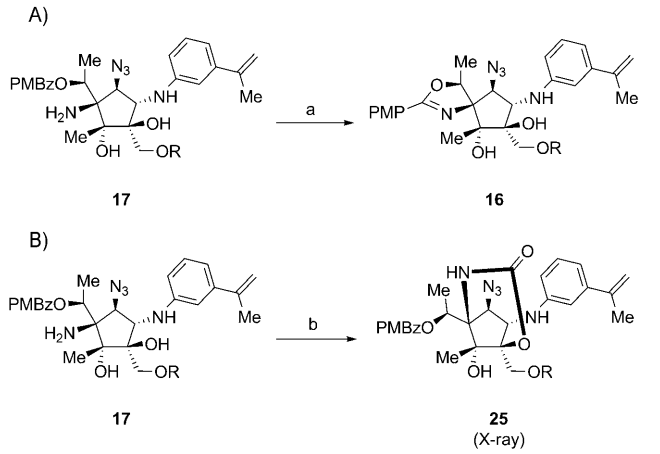


Scheme 5. Synthesis of pactamycin from the epoxide **14**. Reagents and conditions: a) 3-(prop-1-en-2-yl)aniline (**15**), $Yb(OTf)_3$, toluene, 80 °C, 81%, (91% brsm); b) 2 N HCl, THF, RT, 63%, (83% after two cycles); c) TASF, DMF, 0 °C to RT, 95%; d) 2,2-DMP/CH_2Cl_2 (1:5), CSA, 0 °C to RT, 86%; e) $Cl_3COCOCl$, activated charcoal, TEA, THF, −46 °C, then $HNMe_2$, −46 °C to RT, 86%; f) DIBAL-H, CH_2Cl_2, −78 °C, 90%; g) cat. OsO_4, THF/acetone/H_2O (5:5:1), NMO, then $NaIO_4$, THF/H_2O (1:1), RT, 80%; h) TFA/MeCN/H_2O (5:1:1), 0 °C to RT, 85%; i) **21**, K_2CO_3, DMA, RT, 96%; j) Lindlar's cat., H_2, MeOH/EtOH (1:1), 85%. brsm = based on recovered starting material, CSA = 10-camphorsulfonic acid, DMA = *N*,*N*-dimethylacetamide, DMF = *N*,*N*-dimethylformamide, 2,2-DMP = 2,2-dimethoxypropane, NMO = 4-methylmorpholine *N*-oxide, R = TBDPS, PMBz = *p*-methoxybenzoyl, TASF = tris(dimethylamino)sulfonium difluorotrimethylsilicate.

The total synthesis of pactamycin and pactamycate by the route described here was achieved in 29 linear steps and 3.0% overall yield starting with the known oxazoline **2** readily available from L-threonine.[11] The modular introduction of functional groups allows for a great deal of flexibility in the quest for the synthesis of less toxic congeners that maintain their antibacterial and cytotoxic activities.[18] Efforts toward



Scheme 6. Synthesis of pactamycate. Reagents and conditions: a) DIBAL-H, CH_2Cl_2, −78 °C, 89 %; b) $Cl_3COCOCl$, activated charcoal, TEA, THF, −46 °C, 86 %; c) cat. OsO_4, THF/acetone/H_2O (5:5:1), NMO, then $NaIO_4$, THF/H_2O (1:1), RT, 82 %; d) TASF, DMF, 0 °C to RT, 93 %; e) **21**, K_2CO_3, DMA, RT, 98 %; f) Lindlar's cat., H_2, MeOH/EtOH (1:1), 83 %. R = TBDPS.



Scheme 7. Unexpected results arising from the closeness of functional groups on the polysubstituted cyclopentane core structure. Reagents and conditions: a) *N*,*N*-dimethylcarbamoyl chloride, TEA, CH_2Cl_2, RT, 96 %; b) $Cl_3COCOCl$, activated charcoal, TEA, THF, −46 °C, 91 %. R = TBDPS.

these goals are presently being actively pursued in our laboratory.

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