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Preparation and biological evaluation of synthetic and polymer-encapsulated congeners of the antitumor agent pactamycin: Insight into functional group effects and biological activity

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ABSTRACT

The synthesis and biological analysis of a number of novel congeners of the aminocyclopentitol pactamycin is described. Specific attention was paid to the preparation of derivatives at crucial synthetic branch points of the parent structure, and biological assays revealed a number of insights into the source of pactamycin's biological activity. Additionally, the encapsulation of pactamycin and select derivatives into the PRINT© nanoparticle technology was investigated as a proof-of-concept, and evidence of bioactivity modulation through nanoparticle delivery is demonstrated. This work has provided heretofore unrealized access to a large number of novel compounds for further evaluation.

1. Introduction

Pharmaceutical development through organic synthesis remains a critical feature of the drug discovery process.[1] Upon identification of an initial hit via high-throughput screening, a significant amount of structural modification is often required before a lead candidate can be advanced to clinical trials. Natural molecules are often identified as initial hits in these screenings; however, later modification of their complex structures toward the preparation of useful drug molecules can be hindered by the deficiency of a practical and flexible chemical synthesis.[2] As a result, the continued advancement of synthetic organic methodology is critical for facile and flexible drug discovery and development.

Pactamycin (**1**, Fig. 1) is an example of a valuable natural target that has yet to reach its full medicinal potential, at least in part due to its structural complexity. Isolated in 1961 from a fermentation broth of *Streptomyces pactum* var. *pactum* by scientists at the former Upjohn Chemical Co.,[3] pactamycin represents the most complex aminocyclitol antibiotic ever discovered. Researchers at Upjohn showed it to be active against Gram-positive and Gram-negative bacteria as well as against a number of cancer cell lines in vitro.[4] More recent biological studies have demonstrated pactamycin to have potent antiviral (complete inhibition of polio-infected HeLa cells at 10^{-7} M) and antiprotozoal qualities (P.f. K1: IC_{50} = 14.2 nM).[5,4b] Unfortunately, this promising biological profile is hindered by pactamycin's high cytotoxicity against human eukaryotic cell lines (MRC-5: IC_{50} = 95 nM).[5,4b] X-ray crystallographic studies have shown that the source of this activity stems from pactamycin's ability to bind to the 30S ribosomal subunit acting as an RNA dinucleotide mimic.[6] A complex array of H-bonding interactions within the 30S site enables pactamycin to act as a universal inhibitor of translocation. Its impressive biology has attracted the attention of a multidisciplinary field in hopes of transforming pactamycin into a suitable therapeutic (Fig. 1).

In addition to **1**, a number of naturally-occurring structural congeners have been isolated from related *Streptomyces* bacteria, displaying varied bioactivities. 7-Deoxypactamycin (**2**) and jogyamycin (**3**) have shown increased antiprotozoal activity relative to **1**.[7] A third natural analog, pactamycate (**4**), has also been

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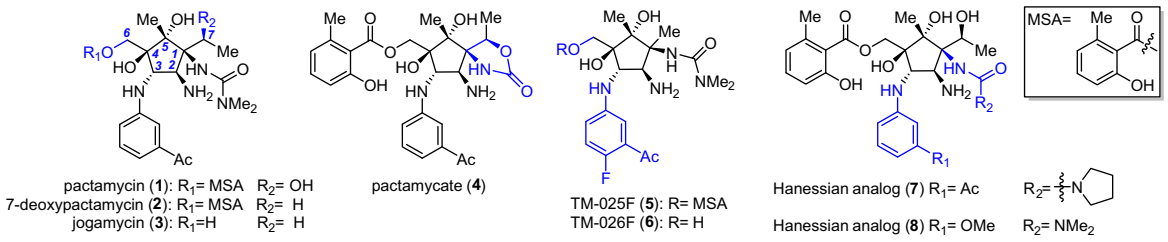

Figure 1. Structures of pactamycin (**1**) and natural, synthetic, and biosynthetic congeners.

reported.[8] Alternatively, biosynthetic engineering studies pioneered by Mahmud and co-workers have provided researchers with the first series of unnatural structural analogs such as TM-025F (**5**) and TM-026F (**6**), which display comparable activities to pactamycin against *Plasmodium falciparum*.[8b,9] These data have renewed promise for pactamycin analogs in drug development.

Moreover, encapsulation of natural cytotoxic agents into nanoparticles (NPs) has also shown improved clinical benefits, the most germane of these being reduction of undesired toxic side effects and increased therapeutic delivery to the target of interest. This approach has been successfully implemented in the case of doxorubicin (Doxil©),[10] paclitaxel (Abraxane©)[11] and others.[12] More recently, Bind Therapeutics[13] and Cerulean[14] have ongoing clinical trials in NP formulations of cancer therapeutics (docetaxel, irinotecan, and camptothecin). DeSimone and co-workers have demonstrated the use of the Particle Replication in Non-Wetting Templates (PRINT®) technology to modulate the activity of cytotoxic agents such as docetaxel, reducing unwanted side-effects and increasing therapeutic activity in vivo.[15] To the best of our knowledge, however, the incorporation of pactamycin or its congeners into NPs of any type with the goal of bioactivity attenuation has not yet been explored.

While an efficient chemical synthesis of **1** might provide the most flexibility in structural derivatization, the inherent complexity of the molecule has rendered this a difficult undertaking. The heavily-compacted and heteroatom-rich functionality in pactamycin presents a number of challenges toward selective structural modification. Additionally, while the unique functional groups present in the molecule (salicylate, dimethylurea, aniline) offer novel branch points for structural diversification, methods with which to install these moieties are underexplored in the literature.[16] To these aims, a number of synthetic studies have been reported by Isobe, Knapp, Looper, Nishikawa, and our group in the past decade.[17] In 2011, Hanessian and co-workers described the first total synthesis of pactamycin in 32 steps from L-threonine, enabling previously unrealized access to synthetic congeners.[18] Since this initial publication, Hanessian has demonstrated the efficacy of his route to deliver pactamycin derivatives at the C1-dimethylurea and the C3 aniline positions such as compounds **7** and **8**.[19]

Our group began work on the total synthesis of **1** in 2009, and this work culminated in 2013 with a 15-step, asymmetric synthesis from commercially available 2,4-pentanedione (Fig. 2).[20] Critical to our approach was to assemble the molecule in a fashion such that key functional groups were installed both in their native form and in a late-stage fashion; we surmised that this approach would provide the greatest possible flexibility, facilitating investigations of structure-activity relationships at all critical branch points. To this end, we envisaged a synthon such as **9** in which exploitation of appropriate functional handles at the correct stage would install the requisite functionalities.

A summary of our disclosed synthesis endgame is described in Figure 3, wherein ketone intermediate **10** (synthesized in ten steps from 2,4-pentanedione in gram quantities) would serve as our first

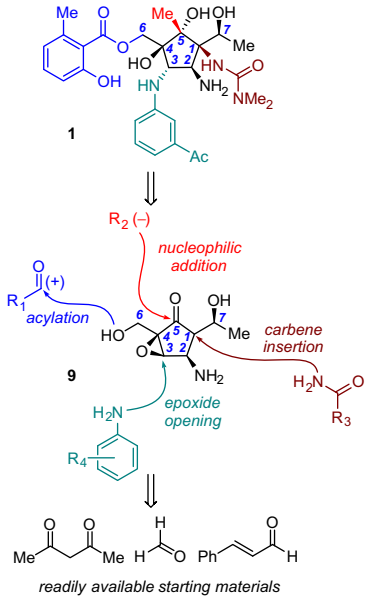

Figure 2. Synthon analysis of **1** showing key branch points for structural derivatization.

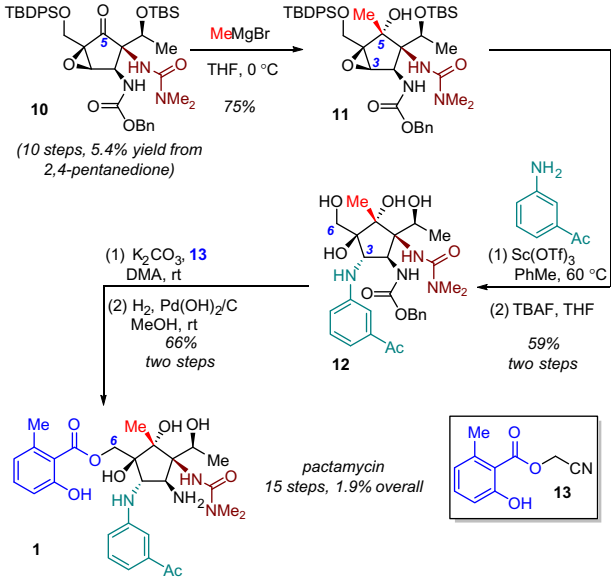

Figure 3. Pactamycin: endgame strategy and synthesis completion.

point of derivatization.[20] Nucleophilic methylation of **10** proceeded in good yield to provide carbinol **11** in 75% yield of a single diastereomer at C5. $Sc(OTf)_3$-promoted addition of *m*-acetylaniline installed the substituted C3-aniline necessary for elaboration to **1**,