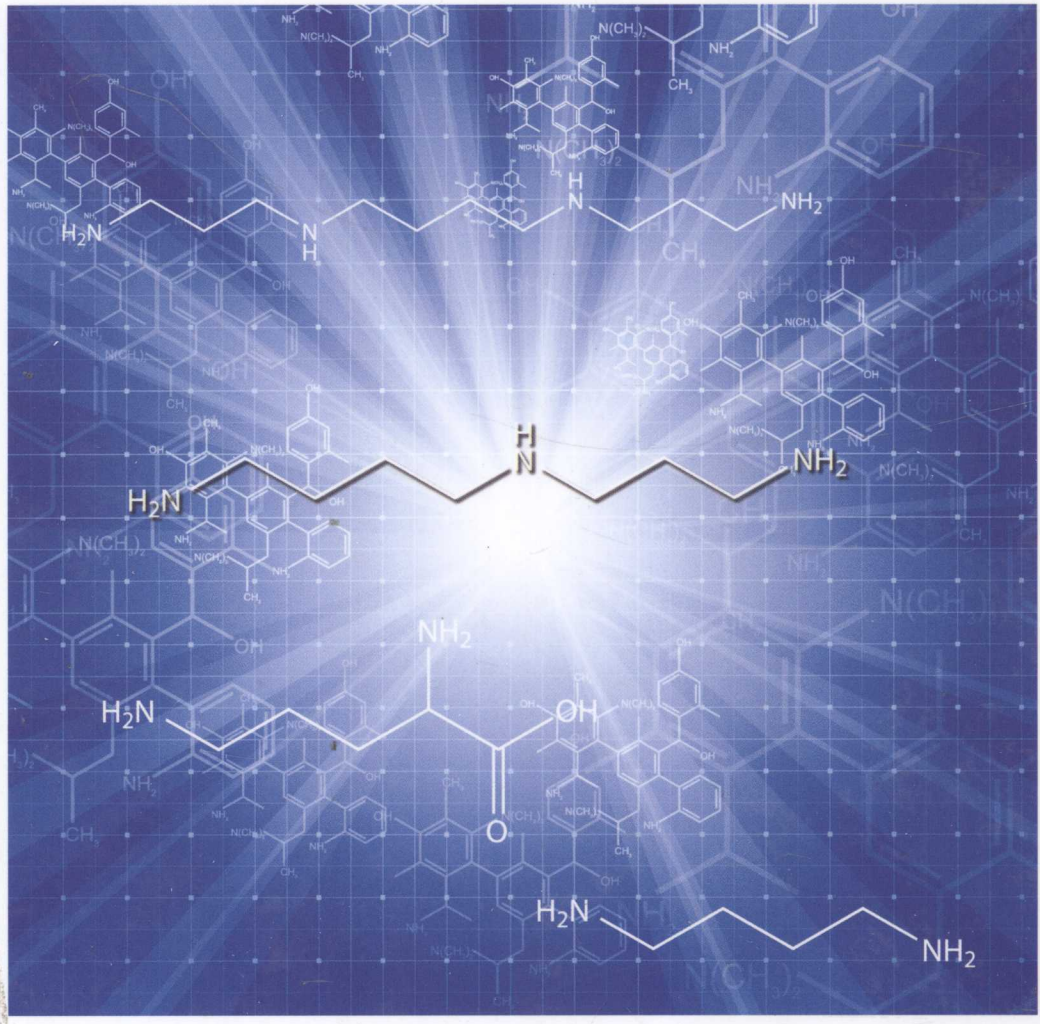
RSC Drug Discovery

Edited by Patrick M. Woster and Robert A. Casero, Jr.

Polyamine Drug Discovery



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Preface

There have been many significant advances in polyamine research since the field was brought to the forefront of biological and biomedical research in the 1970s. Many of the significant findings since that time, whether in plant research, cell biology, the development of therapeutics, genetics or another related field, have been aided by the availability of synthetic compounds specifically designed to inhibit enzymes in the polyamine pathway or otherwise disrupt polyamine metabolism. For example, a search of PubMed using the terms difluoromethylornithine AND dfmo AND eflornithine produces 1179 references dating to 1980, and including research in diverse areas such as plant biochemistry, cancer cell biology, parasitology, insect biochemistry, synthetic chemistry, drug development and human clinical trials. Despite the diversity of fields of endeavor within polyamine research and the significant impact of modulators of polyamine metabolism, a book dedicated to the discovery and development of synthetic compounds targeting polyamine metabolism as drugs has never been produced. The purpose of this book is to fill that void by presenting an overview of drug-discovery research within the polyamine field.

The impetus for a significant portion of polyamine research has been provided by the availability of synthetic analogs that produce defined effects on polyamine metabolism *in vitro* and *in vivo*. This book begins with a chapter that outlines the synthetic approaches to these analogs, covering areas such as nucleotide synthesis and synthetic routes used to access various polyamine analogs. The structural biology aspects of polyamine drug discovery are detailed, as are efforts to design and discover specific inhibitors of enzymes in the polyamine pathway. Chapters are also included that address the role of polyamine analogs as antiparasitic agents, antineoplastic agents and epigenetic modulators. In addition, the important role played by polyamine oxidation is detailed. Other important areas within polyamine drug discovery research, such

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as polyamine transport, the development of polyamine–metal complexes as antitumor agents and the design of polyamine-based gene transfer reagents, are discussed. Finally, a chapter is included that describes the promising results from recent human clinical trials involving drugs targeting polyamine metabolism. The result is a broad overview of polyamine drug discovery and the translation of new chemical entities from basic chemistry to studies involving patients.

Those of us who have a long history in polyamine drug discovery research know that it is a cyclic endeavor, with drug discovery successes appearing periodically and clinical successes appearing steadily but infrequently. However, recent clinical research with existing compounds, including DFMO and the bis(ethyl)polyamine analogs PG-11093 and PG-11144, bode well for the future. In particular, DFMO has found utility as a chemopreventative agent in combination with sulindac, and the bis(ethyl)polyamines have produced promising results in combination antitumor studies. Not since the advent of DFMO has the field of polyamine drug discovery research been so close to bringing a drug to market. We hope that after perusing this book, the reader will have gained an appreciation for polyamine drug discovery efforts that are occurring on multiple fronts. We also hope that you will share the confidence inherent in modern polyamine researchers that significant successes in polyamine drug discovery are on the horizon.

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CHAPTER 1

Polyamine Drug Discovery: Synthetic Approaches to Therapeutic Modulators of Polyamine Metabolism

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1.1 Introduction

In the following chapters, a complete description of the design, bioevaluation and development of modulators of polyamine metabolism is presented. There are numerous synthetic approaches to these inhibitors, and as such a comprehensive review of the chemical literature in this area is beyond the scope of this book. In this chapter, specific examples of synthetic approaches to nucleosides, analogs of the natural polyamines and other agents that affect polyamine metabolism are described. The reader should bear in mind that the literature is replete with alternative strategies for the synthesis of compounds described herein. However, the examples provided will allow the reader to appreciate the vast chemical diversity that is available to medicinal chemists working in the polyamine field.

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1.2 Polyamine Metabolism as a Drug Target

The mammalian polyamine biosynthetic pathway is shown in Figure 1.1.[1,2] Ornithine is converted to putrescine by the action of the enzyme ornithine decarboxylase (ODC). Mammalian ODC, a dimeric enzyme with a molecular weight of about 80 000, is a typical pyridoxal phosphate-requiring amino acid decarboxylase that has been studied quite extensively.[3] ODC is known to be one of the control points in the polyamine biosynthetic pathway, producing a product that is committed to polyamine biosynthesis. The synthesis and degradation of ODC are controlled by a number of factors including degradation assisted by a specific ODC antizyme, a polyamine-induced protein that binds to ODC and promotes ubiquitin-independent degradation by the 26S proteasome.[4] As a result, ODC has a functional half-life of about 10 min. Putrescine is next converted to spermidine via an aminopropyltransferase known as spermidine synthase, which requires decarboxylated *S*-adenosylmethionine as a co-substrate.[5] A second closely related but distinct aminopropyltransferase, spermine synthase, then adds an additional aminopropyl group to spermidine to yield spermine, the longest polyamine occurring in mammalian systems.[5] The by-product for the spermidine and spermine synthase reactions is 5′-methylthioadenosine (MTA), a potent product inhibitor for the aminopropyl transfer process.[6] In mammalian systems, MTA is rapidly hydrolyzed by the enzyme MTA-phosphorylase, and the components are converted to adenosine and methionine *via* salvage pathways.[7] The aminopropyl donor for both aminopropyltransferases is decarboxylated *S*-adenosylmethionine (dc-AdoMet), produced from *S*-adenosylmethionine (AdoMet) by *S*-adenosylmethionine decarboxylase (AdoMet-DC).[8] AdoMet-DC, like ODC, is a highly regulated enzyme in mammalian cells, and also serves as a regulatory point in the pathway. However, unlike ODC, AdoMet-DC belongs to a class of pyruvoyl enzymes that do not require pyridoxal phosphate as a cofactor (see below).[9]

Polyamine metabolism is tightly controlled by a combination of inducible enzymes and the import/export of cellular polyamines. In addition to the enzymes mentioned above, intracellular polyamine content is modulated by a pair of acetyltransferases. Spermidine in the cell nucleus is acetylated on the four-carbon end by spermidine-*N*8-acetyltransferase, possibly altering the compound's binding affinity for DNA.[10,11] A specific deacetylase can then reverse this enzymatic acetylation. Cytoplasmic spermidine and spermine serve as substrates for spermidine/spermine-N^1-acetyltransferase (SSAT), resulting in acetylation on the three-carbon end of each molecule (Figure 1.1).[5,12] The acetylated spermidine or spermine then acts as a substrate for acetylpolyamine oxidase (APAO),[13] which catalyzes the formation of 3-acetamidopropionaldehyde and either putrescine or spermidine, respectively. Excess acetylated polyamines can also be exported from the cell via the polyamine transport system.[14] More recently, a second polyamine oxidase, the inducible spermine oxidase (SMO) was discovered and characterized.[15,16] Thus, SSAT, APAO and SMO together serve as a reverse route for the interconversion of polyamines. An additional mechanism for control of cellular polyamines is provided by the polyamine

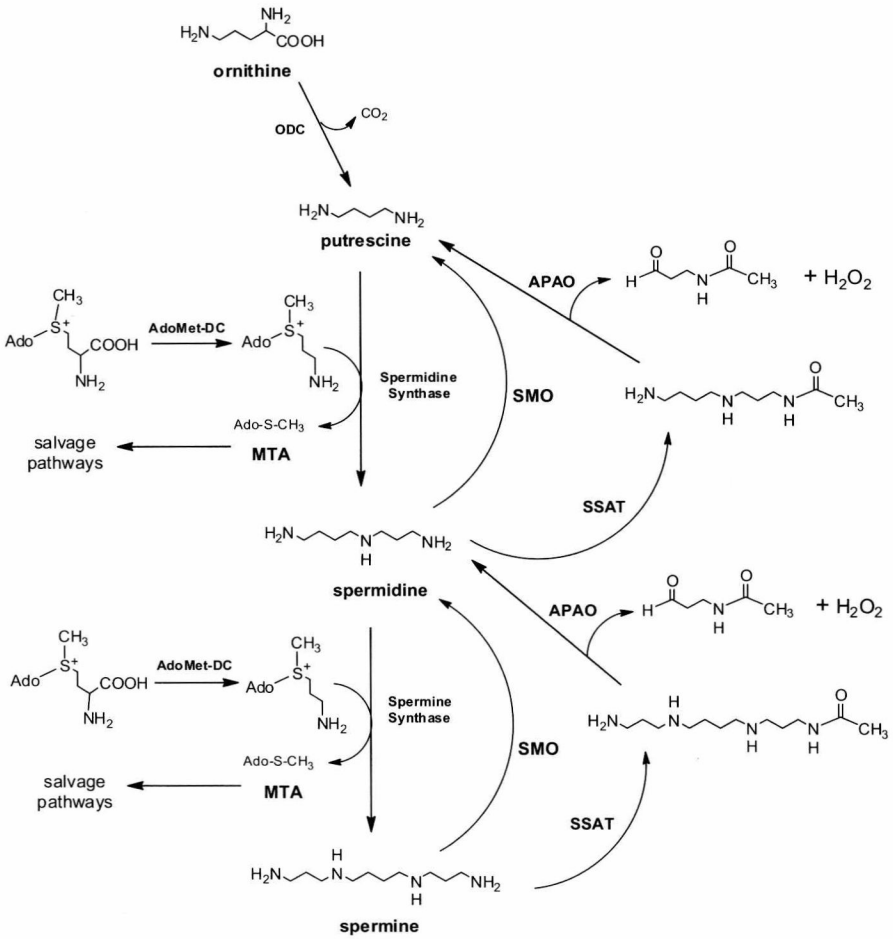


Figure 1.1 Mammalian polyamine metabolic pathway.

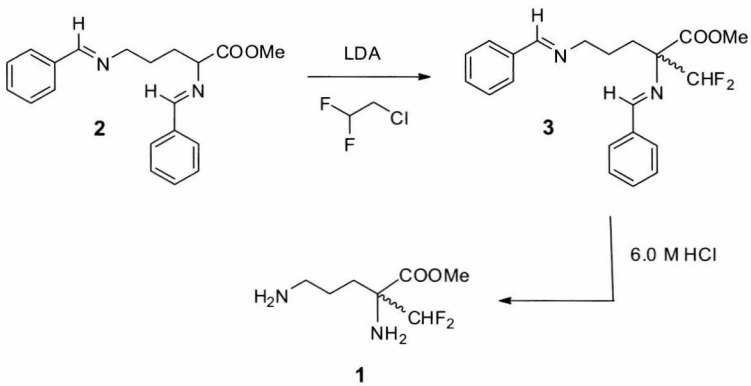
transport system, which has been well characterized in some organisms (bacteria, yeast), but has not been well characterized in mammalian organisms.[17] The function of enzymes in polyamine metabolism and the polyamine transport system, and the consequences of modulating their activity, are described in more detail elsewhere in this book.

1.3 Synthetic Approaches to Modulators of Polyamine Metabolism and Function

1.3.1 Ornithine Decarboxylase (ODC)

Mammalian ODC is a highly unstable protein, and cellular levels of ODC depend on rates of synthesis and degradation as outlined above. For this

reason, reversible and irreversible inhibitors of ODC have proven to be of limited value, since the synthesis of new protein occurs very rapidly in response to reduced polyamine levels in the cell. The catalytic mechanism of ODC involves the formation of a Schiff's base between the amino group of ornithine and the pyridoxal phosphate cofactor which is tightly bound to ODC. The most useful inhibitor of ODC to date, α-difluoromethylornithine (DFMO, **1**, Scheme 1.1), takes advantage of this aspect of the mechanism, and belongs to a group of rationally designed mechanism-based inactivators specifically targeted to individual amino acid decarboxylases. The chemical synthesis of DFMO is shown in Scheme 1.1.[18] The (bis)benzylidene-protected amino ester **2** is treated with lithium diisopropylamide (LDA) followed by exposure to 1-chloro-2,2-difluoroethane to form the alkylated product **3**. Removal of the benzylidene protecting groups and cleavage of the methyl ester are accomplished simultaneously to afford DFMO **1** in a 60% overall yield. It is noteworthy that the pathway shown in Scheme 1.1 is not used at the industrial scale, and the large-scale production of DFMO is an expensive undertaking. Thus, until recently, the drug has been produced almost exclusively in sufficient quantities for inclusion in commercial preparations such as the the lifestyle drug Vaniqa®. Although DFMO is available commercially in small quantities for research, the cost is prohibitive.



Scheme 1.1 Synthesis of 2,2-difluoromethylornithine (DFMO, **1**).

The mechanism of inactivation of ODC by DFMO is shown in Scheme 1.2. As a substrate analog, DFMO forms a Schiff's base with the pyridoxal phosphate cofactor bound to ODC. The subsequent decarboxylation step results in the generation of a latent electrophile, and ODC is rapidly and irreversibly deactivated by forming a covalent bond with CYS_{360}.[19] The discovery of DFMO provided an enormous stimulus to the field of mammalian polyamine biology. Historically, DFMO has been marketed as a treatment for *Pneumocystis carinii* secondary infections in immunocompromised patients,[5] and has been shown to be effective in curing infections of *Trypanosoma brucei gambiense* (but not *T. brucei rhodesiense)* in limited clinical trials.[20–24]