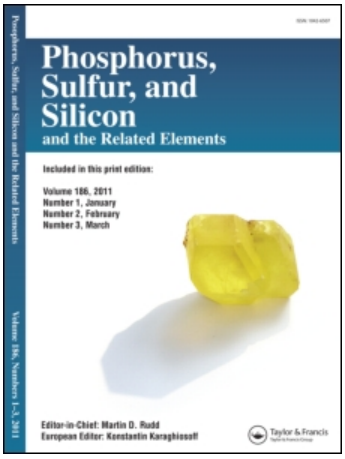

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

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To cite this Article Pankiewicz, Krzysztof W. , Lesiak, Krystyna B. and Watanabe, Kyoichi A.(1999) 'Synthesis of Methylenebis(Phosphonate) Analogues of Nucleotide Coenzymes. A Novel Coupling Mechanism', Phosphorus, Sulfur, and Silicon and the Related Elements, 144: 1, 671 — 674
To link to this Article: DOI: 10.1080/10426509908546334
URL: http://dx.doi.org/10.1080/10426509908546334

Phosphorus, Sulfur and Silicon, 1999, Vol. 144-146, pp. 671-674
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Synthesis of Methylenebis(Phosphonate) Analogues of Nucleotide Coenzymes. A Novel Coupling Mechanism

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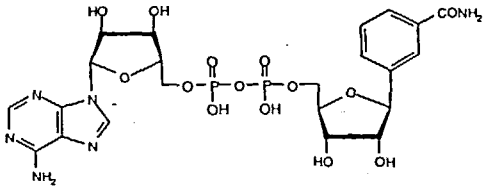
Synthesis of P^1, P^2-disubstituted methylenebis(phosphonate)s as inhibitors of inosine monophosphate dehydrogenase is presented.

Keywords: methylenebis(phosphonate)s; inhibitors of IMPDH

INTRODUCTION

P^1, P^2-Disubstituted pyrophosphates play an important role in a variety of biochemical transformations. Among them NAD and FAD serve as the major electron carriers in biological dehydrogenations. It can be expected that analogues of these compounds, which bind at the appropriate catalytic site of their dependent enzyme but are incapable of carrying out their function, may inhibit the enzyme and eventually exert cytotoxic effects. For example, it has been demonstrated that an NAD mimic, such as benzamide adenine dinucleotide (BAD, Fig. 1) which could not participate in a hydride transfer, was a potent inhibitor of NAD-dependent enzyme, inosine monophosphate dehydrogenase (IMPDH).[1] IMPDH is a key enzyme in *de novo* synthesis of purine nucleosides and is an important target in cancer chemotherapy and immunosuppression since the level of IMPDH activity is markedly increased in cancer cells and activated



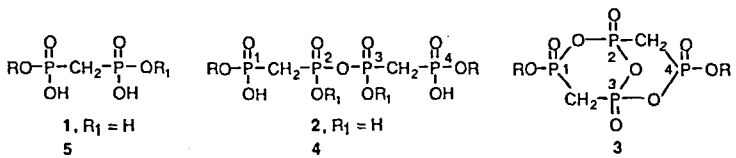
BAD

FIGURE 1

lymphocytes. Pyrophosphates such as BAD, however, are useless as potential drugs since they do not penetrate cell membrane and are metabolically unstable. In contrast, methylenebis(phosphonate) analogues (MBPs) are stable and capable of crossing cell membranes. They resemble well the shape and size of the natural pyrophosphates and may be of chemotherapeutic interest. However, there are no efficient methods for the synthesis of P^1, P^2-disubstituted MBPs.

CHEMICAL SYNTHESIS and BIOLOGICAL RESULTS.

Recently we discovered[2] that nucleoside 5'-MBPs (**1**, Fig. 2) undergo reaction with DCC forming the corresponding tetraphosphonate **2** which is further dehydrated to give the bicyclic trisanhydride **3**. Since **3** is neutral, it readily reacts with nucleophilic reagents preferentially at P^2 and P^3 due to steric hindrance of bulky nucleoside groups at P^1 and P^4. Thus, reaction of **3** with a number of nucleosides, sugars, and alcohols afforded P^2,P^3-disubstituted tetraphosphonates **4**, which upon hydrolysis, gives the desired disubstituted MBPs **5** in good yield.



1, R_1 = H
5

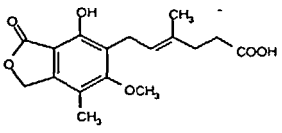
2, R_1 = H
4

3

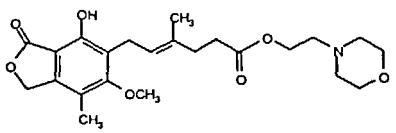
FIGURE 2

When 2-(4-nitrophenyl)ethyl MBP was converted into **3** (R = 4-nitrophenylethyl) and nucleosides were used as nucleophilic reagents, the corresponding nucleoside 5'-MBPs **4** substituted with the nitrophenylethyl group were obtained. Removal of the nitrophenylethyl group by ß-elimination with DBU afforded the desired nucleoside 5'-

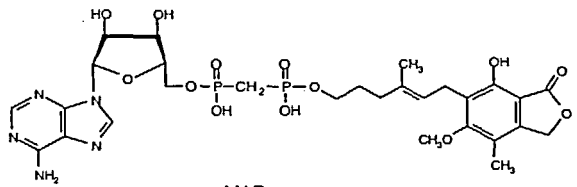
MBPs (1, R = H) which could be further converted into the corresponding P^1, P^2-disubstituted MBPs[3]. Using our new methods we synthesized MBP analogues of TAD, BAD, FAD, ADP-ribose, CDP-dipalmitoylglycerol, and CDP-aminoethanol.[1]



MPA



MPA-mofetil



MAD

FIGURE 3

Mycophenolic acid (MPA, Fig. 3), an antibiotic discovered in the end of the last century, is the most potent inhibitor of IMPDH. Two isoforms of human IMPDH are known. Type I is expressed constitutively while the levels of type II are markedly increased in tumor cells and activated lymphocytes. Among known inhibitors of IMPDH only MPA exerts better affinity against the type II isoform (K_i = 6-10 nM) than type I (K_i = 33-37 nM)[5]. MPA is also very specific; inhibition of another human enzyme by MPA has not been reported. In the form of a prodrug, mycopenolic mofetil (Fig. 3), it is now used in clinics as immunosuppressant. MPA, however, is inactive against tumors because it is quickly converted into the inactive ß-glucuronide. In humans as much as 90% of the drug circulates in this inactive form. MPA inhibits proliferation of B and T lymphocytes probably because the glucuronidation activity is low or vanished in lymphocytes.

Recently an X-ray structure of the complex of a Chinese Hamster IMPDH with IMP and MPA has been solved.[6] It was found that MPA binds to the cofactor site of IMPDH resembling the binding of nicotinamide mononucleotide (NMN) moiety of NAD. Therefore, MPA can be considered as a mimic of NMN. Consequently,

coupling of MPA with adenosine 5'-monophosphate (AMP) should result in formation of NAD analogue, mycophenolic adenine dinucleotide (MAD). We synthesized an MBP analogue of MAD (Fig. 3) using mycophenolic alcohol obtained by reduction of the carboxylic group of MPA with $NaBH_4$. The MBP analogue of MAD was found to be a potent inhibitor of IMPDH as well as growth of K562 cells in culture.[4] In contrast to MPA, MAD was not converted into the glucuronide by uridine 5'-diphosphoglucuronyltransferases from bovine liver microsomal preparation. Since glucuronidation is known to be one of defending mechanisms in a variety of cancer cells, MAD and its analogues may be of potential therapeutic interest as anticancer agents. It is also possible that due to resistance to glucuronidation these compounds may serve as improved and potent immunosuppressants. Synthesis of new MAD analogues and their biological activity will be discussed.

Acknowledgment

The authors thank Dr. Takashi Tsuji of Aijnomoto Co., Inc., Japan, for a gift of MPA.

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