Acta Biochimica Polonica

Vol. 43 No. 1/1996

183–194

QUARTERLY

This paper is dedicated to Professor David Shugar on the occasion of his 80th birthday

NAD-analogues as potential anticancer agents: Conformational restrictions as basis for selectivity*

Krzysztof W. Pankiewicz[a**], Andrzej Zatorski[b] and Kyoichi A. Watanabe[a]

[a]*OncorPharm, 200 Perry Parkway, Gaithersburg, MD 20877, U.S.A.*
[b]*Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, Sloan-Kettering Division of Graduate School of Medical Sciences, Cornell University, New York, New York 10021, U.S.A.*

Key words: NAD-analogues, inhibitors of IMP-dehydrogenase, tiazofurin, anticancer agents

Cofactor type inhibitors (NAD-analogues) of IMP-dehydrogenase (IMPDH) were synthesized and their application as potential anticancer agents are discussed. C-nucleoside isosteres of NAD, C-NAD and C-PAD, showed an effective competitive inhibition of IMPDH. C-NAD but not C-PAD caused extremely potent inhibition of alcohol dehydrogenase. We also synthesized compounds in which nicotinamide riboside was replaced with tiazofurin (TAD-analogues) and the 2′ and 3′-positions of adenosine part were fluorinated. The ribose ring of 2′-deoxy-2′-fluoroadenosine is in the C3′-*endo* conformation whereas 3′-deoxy-3′-fluoroadenosine favors the C2′-*endo* sugar pucker. These derivatives are good inhibitors of IMPDH type II, the isoenzyme dominant in neoplastic cells. In contrast, all these analogues showed rather week inhibitory activity against alcohol dehydrogenase. Nicotinamide riboside derivatives in which the base and the sugar are linked through an oxygen or a methylene bridge were synthesized. NAD-analogues containing such conformationally restricted nicotinamide nucleoside moiety (*syn* or *anti*) are expected to be selective inhibitors of B-specific (IMPDH) or A-specific dehydrogenases, respectively.

IMP-dehydrogenase (IMPDH), the enzyme which catalyzes the NAD-dependent conversion of inosine 5′-monophosphate to guanosine 5′-monophosphate, is positioned at the branch point in the *de novo* synthesis of guanine nucleotides [1]. Inhibition of IMPDH has been shown to have antiviral [2, 3], immunosuppressive [4, 5], antiparasitic [6, 7] and anticancer effects [8, 9]. IMPDH has been suggested [1, 8, 9] as a key enzyme in neoplasia and one of the most sensitive targets for cancer chemotherapy. The level of IMP-dehydrogenase activity was found to be much greater in several tumors as compared to normal tissues [10]. Human IMPDH cDNA was first cloned and sequenced by Collart & Huberman [11] showing that the protein contained 514 amino acids. Increased IMPDH gene expression has been then found in several tumor cell lines [12]. It has been recently discovered [13] that human IMP-dehydrogenase exists as two isoforms, types I and II. The two distinct cDNAs have been characterized and it has been found that the two isoforms are differently regulated [14–16]. Type I is constitutively ex-

*This research was supported by grant from National Institute of General Medical Sciences, National Institutes of Health and Human Services (Grant No. GM 42010 (KWP)).

**To whom correspondence should be addressed.

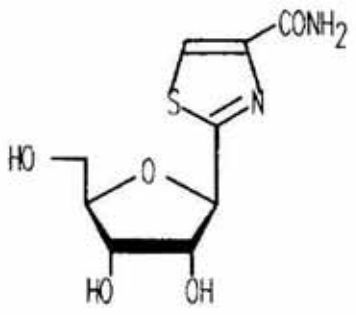
Abbreviations: ADH, alcohol dehydrogenase; C-NAD, C-glycosidic nicotinamide adenine dinucleotide; C-PAD, C-glycosidic picolinamide adenine dinucleotide; IMP, inosine 5′-monophosphate; IMPDH, IMP-dehydrogenase; TAD, thiazole-4-carboxamide adenine dinucleotide.

pressed and is the preponderant isoform in normal cells, while type II is selectively up-regulated in neoplastic and replicating cells and emerges as the dominant species. Interestingly, when neoplastic cells are induced to differentiate, the type II transcript is selectively down-regulated to a level below that of type I. Thus, the selective inhibition of type II IMPDH is expected to provide significant therapeutic advantage by eliminating or reducing potential toxicity caused by inhibition of type I isoform [13–16].

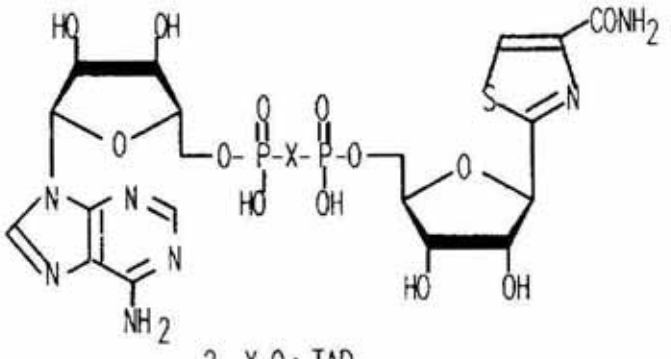
Tiazofurin, 2-(β-D-ribofuranosyl)thiazole-4-carboxamide, is metabolically converted into the NAD analogue, thiazole-4-carboxamide adenine dinucleotide (TAD, Scheme 1) which was found to be a competitive inhibitor of IMP-dehydrogenase [17]. Tiazofurin exhibits potent activity against various murine and human tumors [18, 19], and has been evaluated as an anticancer agent in Phase 1 and Phase 2 clinical studies [20]. In recent years, the ability of tiazofurin to decrease guanine nucleotide pool created new hope and excitement. In 1991 the compound was on the cover of the journal *Blood* [21, 22] as an inducer of myeloid differentiation. Due to the inhibition of G-protein mediated transmembrane signaling [23], cells had undergone maturation and differentiation. Weber [24] noted that patients with chronic granulocytic leukemia in blast crisis treated with tiazofurin demonstrated no depletion in bone marrow. Examination of bone marrow showed decrease in blast cells and increase in more mature forms of granulocytes. These results appear to indicate drug-induced cell differentiations in humans [24–27]. More importantly this treatment has induced complete remissions in 77% of patients [24]. It was also found that tiazofurin treatment modulates gene expression. Rapid decrease in c-Ki-*ras* messenger RNA levels and *c-myc* expression in K-562 cells were reported, whereas the mRNA level of *c-abl* gene was not affected [27–29].

Tiazofurin requires a unique metabolic activation. It is phosphorylated by adenosine kinase or other kinase(s) to the 5′-monophosphate [30] and then converted to TAD by NAD-pyrophosphorylase [31]. TAD has been synthesized [31, 32] and has been found to be an even more potent inhibitor of IMP-dehydrogenase than tiazofurin. TAD mimics NAD but can not function as the coenzyme. It was found to interfere with NAD synthesis *in vitro*, and is a weak inhibitor of poly(ADP-ribose) synthase [33]. Accumulation of TAD, however, is regulated by cellular phosphodiesterase called TAD-ase, which is responsible for TAD breakdown and development of cell lines resistant to tiazofurin [34, 35]. In order to circumvent the resistance development, a TAD analogue containing the phosphonate (-P-CH_2-P-, Scheme 1) instead of pyrophosphate (-P-O-P-) moiety was synthesized [36]. This analogue (β-CH_2-TAD) is not susceptible to enzymic hydrolysis, and is the most potent inhibitor of IMP-dehydrogenase [36].

Since the discovery of oncolytic properties of tiazofurin, other C-nucleoside analogues have been studied. Selenazofurin (**5**, Scheme 2), an analogue containing selenium instead of sulfur, was synthesized [37] and its corresponding dinucleotide anabolite was found to be even more cytotoxic than tiazofurin. In contrast, isosteric replacement of the sulfur or selenium with oxygen, as in oxazofurin (**6**) [38] abolished the antitumor activity. Arabino (**7**) and xylo (**8**) congeners of tiazofurin show no cytotoxicity



1. Tiazofurin



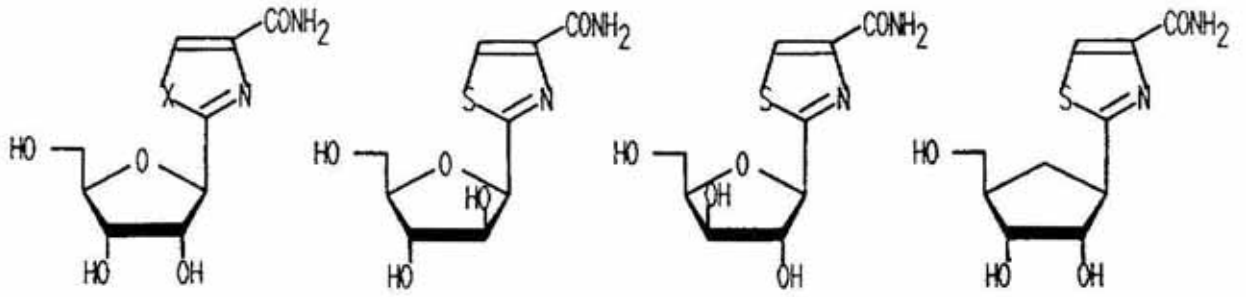
2. X=O; TAD
3. X=CH_2; β-methylene TAD
4. X=CF_2; β-difluoromethylene TAD

Scheme 1

[39]. Recently, carbocyclic tiazofurin (**9**) and its enantiomer have been reported to display cytotoxicity against a breast carcinoma cell line [40]. Another nucleoside, the 5-(β-D-ribofuranosyl)-1,2,4-oxadiazole-3-carboxamide (**10**, Scheme 3) caused only weak inhibition of leukemia L1210 and P388 lines [41]. No activity was reported for 1,3,4,-thiadiazole C-nucleoside analogue **11** of tiazofurin [42]. An imidazole C-nucleoside, 4-(2-deoxy-β-D-ribofuranosyl)imidazole-2-carboxamide (**12**) has also been prepared recently [43]. The synthesis of the 2-(β-D-ribofuranosyl)pyrimidine-4-carboxamide (**13**) and its NAD analogue has been published, but the antitumor activity of these compounds has not been reported [44].

Two novel, potent inhibitors of IMPDH have recently been reported. First, 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (**14**, Scheme 4, synthesized by Matsuda *et al.* [45]) and its 5'-monophosphate derivative inhibit IMPDH from L1210 cells competitively with respect to IMP [46]. Second, 3-β-D-ribofuranosylbenzamide (**15**) showed toxicity in a nanomolar concentration to S49.1 lymphoma cells [47] and leukemia K562 cells [48]. Interestingly, benzoic acid riboside is almost nontoxic to the lymphoma cells [47]. Benzamide adenine dinucleotide has been prepared enzymatically and found to be an active metabolite of benzamide riboside [49].

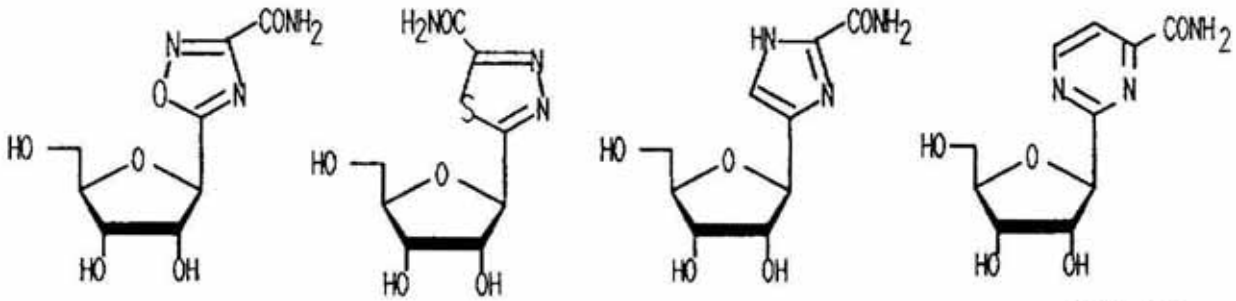
The known inhibitors of IMPDH can be broadly classified into three groups based upon the mode of enzyme binding [50]. The first group consists of IMP/XMP (substrate) analogues such as 6-chloropurine ribotide (**16**, Scheme 5), riba-



5 X = Se
6 X = O

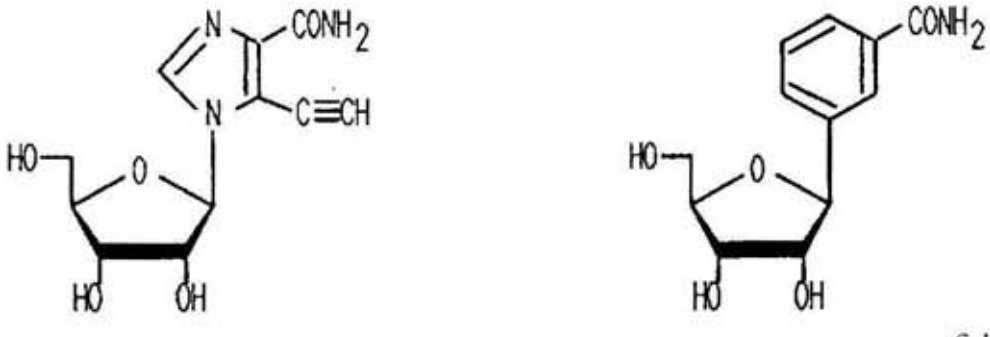
7 8 9

Scheme 2



10 11 12 13

Scheme 3



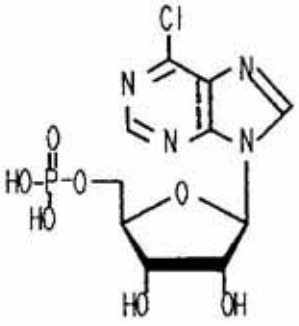
14 15

Scheme 4

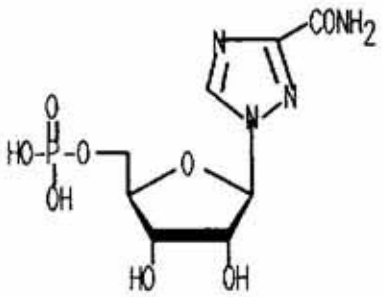
virin-5'-phosphate (**17**), and bredinin-5'-phosphate (**18**). The second group are the NAD^+/NADH analogues, tiazofurin and mycophenolic acid (**19**). The third group of IMPDH inhibitors are 2-thiobenzyl IMP (**20**) and 8-thiobenzyl AMP (**21**), multisubstrate inhibitors that presumably occupy the IMP/XMP binding domain with groups projecting into NAD site to enhance binding. The importance of cofactor type inhibitors consists in their mode of action which offers specificity for IMPDH [50]. More general inhibitors of NAD-dependent enzymes would be expected to have greater toxicity [50].

We have reported [51–53] the synthesis of the closest structural analogues of nicotinamide riboside, i.e. C-nucleoside isosteres — nicotinamide **22** and picolinamide **23** (Scheme 6). Later, 2-β-D-ribofuranosyl-isonicotinamide (**24**) was synthesized by others [54]. In contrast to our expectation these compounds showed only weak inhibitory activity against L1210, P-815, HL-60, CCRF-CEM, MOLT/4F, and MT-4 cell lines [51–54].

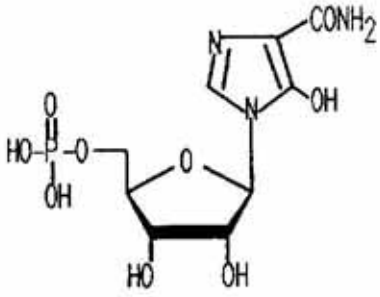
Recently, we converted our nicotinamide C-nucleoside **22** and picolinamide C-nucleoside **23** into the corresponding NAD analogues, C-NAD (Scheme 7) and C-PAD, respectively [55]. We found [55] that C-NAD was an effective competitive inhibitor of IMPDH (ID_{50} = 20 μM). Interestingly, C-NAD caused extremely potent inhibition of horse liver alcohol dehydrogenase (ADH, K_i = 1.1 nM), whereas C-PAD was found to be a much less potent inhibitor of ADH



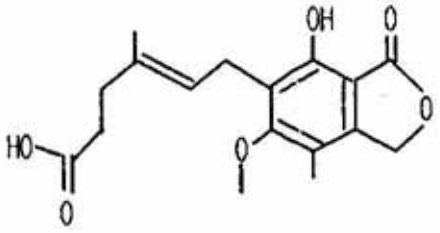
16



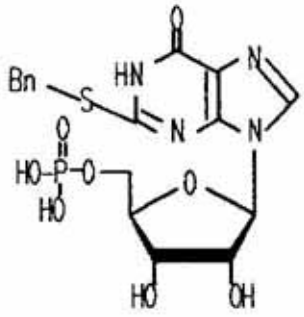
17



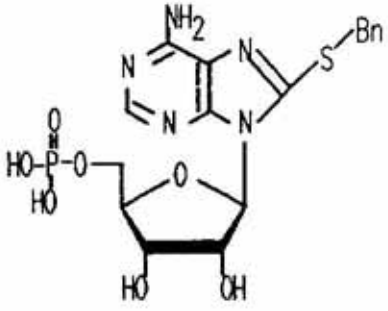
18



19

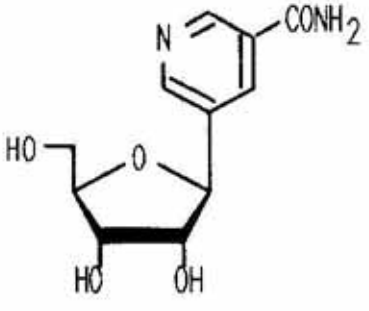


20

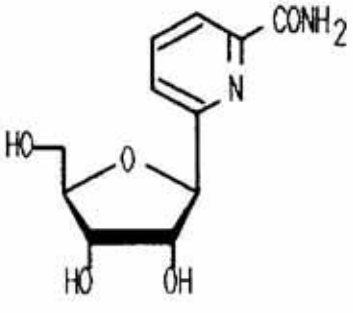


21

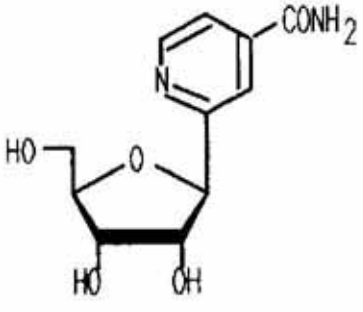
Scheme 5



22



23



24

Scheme 6

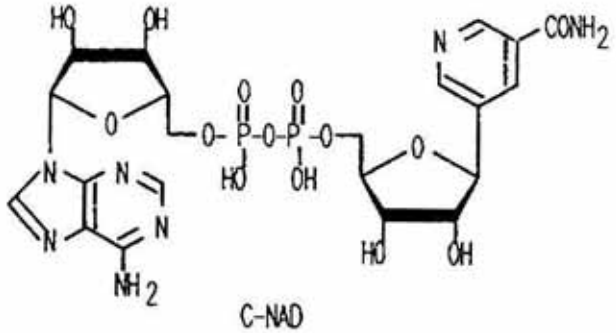
(K_i = 20 μM). It was later discovered by Goldstein *et al.* [56, 57] that the specificity and affinity of C-NAD for ADH are due to coordination of the zinc cation at the ADH catalytic site by the pyridine nitrogen of C-NAD. Displacement of the pyridine nitrogen to the opposite side of the ring, as in C-PAD, removes the specificity for ADH [56, 57].

All these studies indicate that with the exception of tiazofurin, selenazofurin, and perhaps carbocyclic tiazofurin, other nucleosides related to nicotinamide riboside are not efficiently converted into corresponding NAD analogues by cellular enzymes. Therefore, NAD analogues, *not nucleosides*, that are able to penetrate cells membrane should be of therapeutic interest. These NAD analogues should be specific inhibitors of IMPDH (possibly type II), not toxic or less toxic towards cellular dehydrogenases. In contrast to nucleosides, NAD analogues do not require metabolic activation by cellular enzymes. NAD analogues containing C-nucleosides instead of nicotinamide riboside should be resistant to cellular catabolic enzymes, such as NAD-glycohydrolases. Methylene bisphosphonate analogue of NAD should be effective against tiazofurin resistant cell lines [34, 35] due to "built in" resistance to NAD-phosphodiesterases.

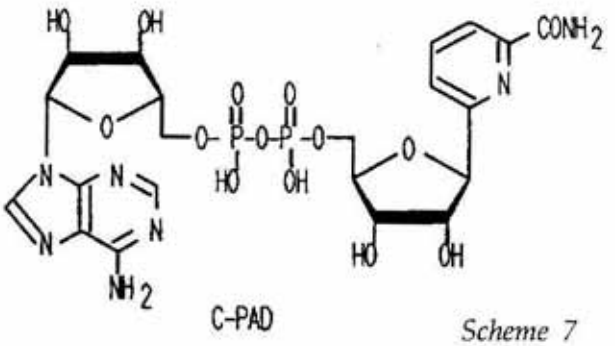
Since the X-ray structure of IMPDH is not known, we used well established ADH structure for general studies of enzyme-inhibitor (cofactor type) interactions. Our recent crystallographic studies [58] of a complex of ADH and β-methylene-TAD showed preservation of hydrogen bonds between donors O2′ and O3′ of the adenosine ribose and negatively charged Asp-223. In addition, the positively charged Lys-228 donates a hydrogen bond to adenosine O3′. These interactions are also observed in ADH-bound NAD, and are conserved in a number of dehydrogenases [59]. In IMPDH, as in ADH, TAD displaces NAD binding at the cofactor site. Initial recognition of the cofactor appears to occur *via* binding to the adenosine end [60].

These findings prompted us to synthesize three TAD analogues containing fluorine atom at the C2′-*ribo*, C3′-*ribo*, and C2′-*arabino* positions of the adenine nucleoside (**25**, **26**, and **27**, respectively, Scheme 8) as probes of the stereochemical requirements of the adenine end on IMPDH [61]. Fluorine can mimic hydroxyl group in terms of size and polarity [62] and is able to form an even stronger hydrogen bond than a hydroxyl group, acting as an acceptor. Some dehydrogenases require the C2′-*endo* conformation of the adenosine ribose, others require C3′-*endo*. Interestingly, the ribose ring of 2′-deoxy-2′-fluoroadenosine is in a C3′-*endo* conformation due to the highly electronegative 2′-substituent [63]. In contrast, 3′-deoxy-3′-fluoroadenosine favors a C2′-*endo* sugar pucker [64]. Thus, TAD analogues **25**–**27** are expected to bind tightly to enzymes which are able to provide a good donor type hydrogen bonding.

We found [61] (Table 1) that all our compounds showed good inhibitory activity against IMPDH-II, the isoenzyme predominant in neoplastic cells [16]. Analogues **25** (K_i = 0.5 μM), **26** (K_i = 0.7 μM), and **27** (K_i = 2.9 μM) were a little less effective than TAD (K_i = 0.2 μM). Interestingly, the level of inhibition of ADH was found to be much lower (0.1 mM for **25** and **26**, and no inhibition up to 10 mM for **27**). Thus, new compounds are potent inhibitors of IMPDH-II, show some selectivity, and may be of therapeutic potential. TAD-**25** and TAD-**27** do not contain the 2′-hydroxyl function of the adenosine moiety and therefore can not be converted into the corresponding NAD(P) analogue by cellular enzyme(s). Thus, these compounds are expected to be less toxic than TAD itself, since the former should be



C-NAD



C-PAD

Scheme 7

harmless toward numerous NAD(P)-dependent cellular enzymes.

We also synthesized the β-difluoromethylene-TADs (β-CF_2-TADs, **28** and **29**, Scheme 9) [65] containing -CF_2- group as replacement of pyrophosphate oxygen, and found [61] that β-CF_2-TAD-**28** (K_i = 0.17 μM) was almost equally effective to β-CH_2-TAD (K_i = 0.11 μM) against IMPDH-II. Transport properties, metabolism, and toxicity of these fluoro-substituted analogues are to be studied.

We assumed that even subtle modifications in the structure of inhibitor of cofactor type, might alter selectivity against targeted dehydrogenases. Thus, changes in conformation of adenine sugar moiety, *vide supra*, or restrictions in *syn - anti* positioning of nicotinamide (rotation around the glycosyl bond), *vide infra*, should affect drug-enzyme interactions.

Restricted rotation around the glycosyl bond of tiazofurin has been suggested as an important factor in its biological activity [66]. It was speculated that good IMPDH inhibitory activity of tiazofurin was due to its favorable conformation for binding to enzyme(s) that converts tiazofurin to TAD, or due to tight binding of TAD to IMPDH. Goldstein and his group found that close intramolecular contact between the sulfur and the ribose ring oxygen in tiazofurin (responsible for restriction in rotation) [66] is maintained in β-CH_2-TAD bound to ADH [58]

The importance of rigid molecules that have fixed glycosyl torsion angle X is probably most dramatically expressed in the case of uridine phosphorylase inhibition. The K_i value of the 2,2'-anhydro-5-ethyluridine, one of the most potent inhibitors of this enzyme, is more than three orders of magnitude lower than that of arabinofuranosyl-5-ethyluracil, the corresponding unrestricted analogue [67].

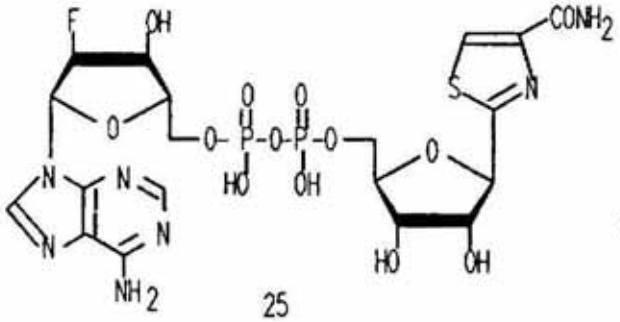
Table 1

Inhibition of human IMP-dehydrogenase type II by TAD analogues.

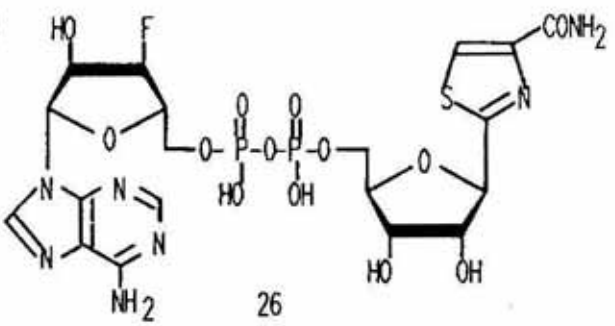
NAD is the variable substrate, with IMP constant at 100 μM. The pattern of inhibition in each case is non-competitive. Values were obtained at 37 °C.

Inhibitor	K_{ii} (μM)	K_{is} (μM)
25	0.5 ± 0.1	6.0 ± 12.0
26	0.7 ± 0.1	1.5 ± 0.6
27	2.9 ± 0.5	13 ± 10
β-CF_2-TAD	0.17 ± 0.03	0.3 ± 0.2
β-CH_2-TAD	0.11 ± 0.02	0.2 ± 0.1
TAD	0.19 ± 0.03	0.3 ± 0.1
NADH	120 ± 7	175 ± 22

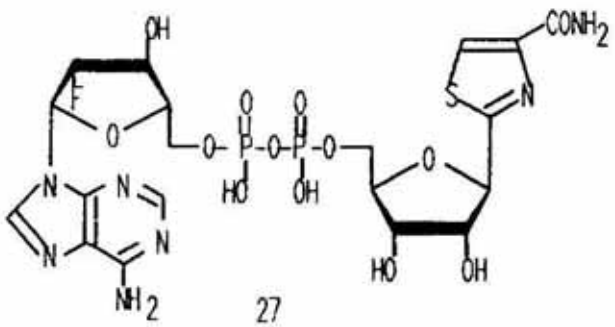
ADH inhibition: K_i's for **25**, **26** and β-CF_2-TAD no less than 0.1 mmol; for **27** no inhibition up to 10 mmol.



25



26



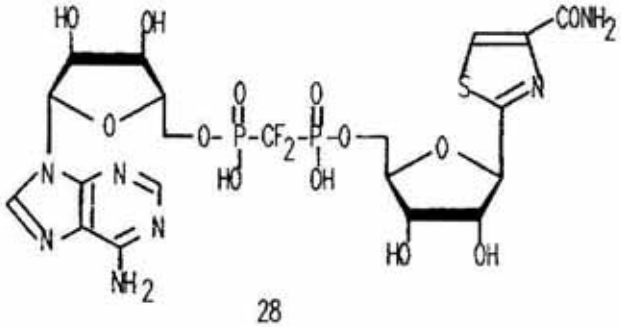
27

Scheme 8

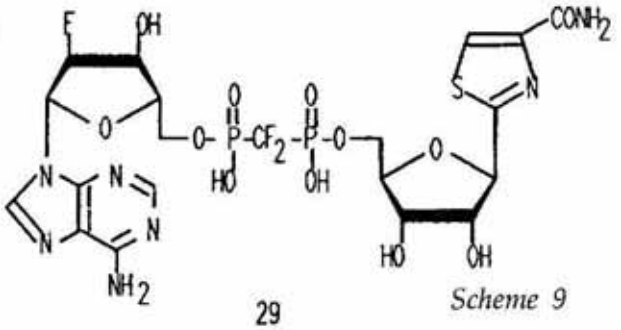
Enzymic oxidation-reduction by all known dehydrogenases are stereospecific processes [68], i.e., only pro-*R* hydrogen of the dihydropyridine ring in NADH is transferred by some dehydrogenases (A-specific, as ADH), whereas others (B-specific, as IMPDH) transfer exclusively pro-*S* hydrogen. The X-ray studies of various complexes of dehydrogenases and NAD revealed that those enzymes that bind the NADH molecule in *syn* conformation transfer pro-*S* hydrogen, whereas transfer of pro-*R* hydrogen requires that NADH is bound to the enzyme in the *anti* conformation [69]. If conformation of nicotinamide nucleoside moiety of NAD analogue is restricted to *syn*, such NAD analogue may serve as coenzyme only for B-specific but not for A-specific dehydrogenases. Therefore, at least in principle, the *syn* conformationally restricted NAD analogues are expected to function as inhibitors only for B-specific enzymes. Consequently, NAD analogue containing *anti* restricted nicotinamide nucleoside should exibit inhibitory activity against A-specific dehydrogenases. Thus, it should be expected that the value of X (defined as the angle O4', C1', N1, C2, with *syn* and *anti* as the borderlines) would dramatically affect enzyme-inhibitor interaction and consequently the selectivity of enzymes inhibition.

In order to test the above hypothesis, we synthesized 2,2'-anhydro-2-hydroxy- and 6,2'-anhydro-6-hydroxy-1-(β-D-arabinofuranosyl)nicotinamide (**30** and **31**, respectively, Scheme 10) [70]. Our attempted conversion of **30** and **31** into their corresponding 5'-monophosphates was, however, unsuccessful due to instability of the anhydro linkage of **30** and **31** resulting in the formation of *arabino* derivatives **32** and **33** [70].

Recently, we have prepared analogues of nicotinamide mononucleotide in which the base and sugar are linked through a methylene bridge, **34** and **35**, making the conformation restricted to the *syn* and *anti*, respectively [71, 72]. We found, however, that due to severe strain in their rigid

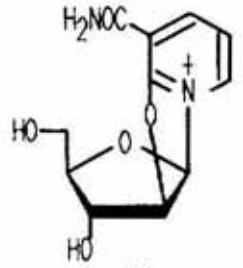


28

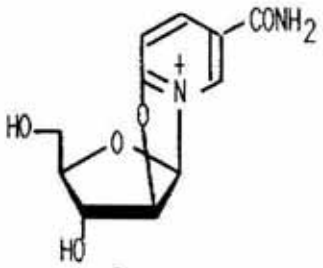


29

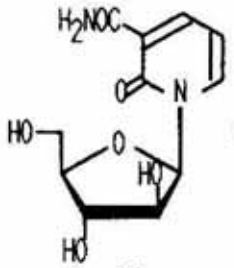
Scheme 9



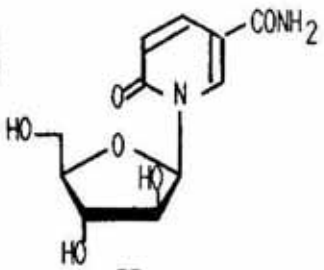
30



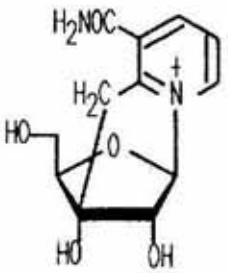
31



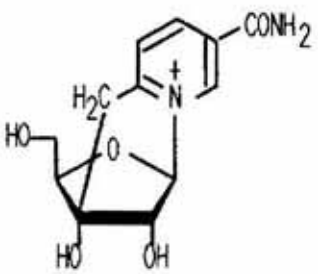
32



33



34



35

Scheme 10

structures, these compounds were susceptible to hydrolysis of the glycosyl bond. Therefore, we are now synthesizing conformationally restricted C-nucleosides with stable glycosyl bond. If the concept of conformationally restricted NAD analogous is valid, approximately half of the cellular dehydrogenases (pro-*S* or pro-*R*) will be unaffected (lack of toxicity) by our synthetic targets.

REFERENCES

1. Weber, G. (1983) Biochemical strategy of cancer cells and the design of chemotherapy: G.H.A. Clowes Memorial Lecture. *Cancer Res.* **43,** 3466–3492.
2. Agbaria, R., Mullen, C.A., Hartman, N.R., Cooney, D.A., Hao, Z., Blease, R.M. & Johns, D.G. (1994) Effects of IMP dehydrogenase inhibitors on the phosphorylation of Ganciclovir in MOLT-4 cells before and after herpes simplex virus thymidine kinase transduction. *Mol. Pharmacol.* **45,** 777–782.
3. Hartman, N.R., Ahluwalia, G.S., Cooney, D.A., Mitsuya, H., Kageyama, S., Fridland, A., Broder, S. & Johns, D.G. (1991) Inhibitors of IMP dehydrogenase stimulate the phosphorylation of the anti-human immunodeficiency virus nucleosides 2′,3′-dideoxyadenosine and 2′,3′-dideoxyinosine. *Mol. Pharmacol.* **40,** 118–124.
4. Morris, R.E. & Wang, J. (1991) Comparison of the immunosuppressive effects of mycophenolic acid and the morpholinoethyl ester of mycophenolic acid (RS-61443) in recipients of heart allografts. *Transplant. Proc.* **23,** 493–496.
5. Nelson, P.H., Eugui, E., Wang, C.C. & Allison, C. (1990) Synthesis and immunosuppressive activity of some side-chain variants of mycophenolic acid. *J. Med. Chem.* **33,** 833–838.
6. Hedstrom, L. & Wang, C.C. (1990) Mycophenolic acid and thiazole adenine dinucleotide inhibition of *Tritrichomas foetus* inosine 5′-monophosphate dehydrogenase: Implication on enzyme mechanism. *Biochemistry* **29,** 849–854.
7. Wilson, K., Collart, F.R., Huberman, E., Stringer, J.R. & Ullman, B. (1991) Amplification and molecular cloning of the IMP dehydrogenase gene of *Leishmania donovani*. *J. Biol. Chem.* **266,** 1665–1671.
8. Jackson, R.C., Weber, G. & Morris, H.P. (1975) IMP dehydrogenase, an enzyme linked with proliferation and malignancy. *Nature (London)* **256,** 331–333.
9. Robins, R.K. (1982) Nucleoside and nucleotide inhibitors of inosine monophosphate dehydrogenase (IMP) as potential antitumor inhibitors. *Nucleosides & Nucleotides* **1,** 35–44.
10. Weber, G., Prajda, N. & Jackson, R.C. (1976) Key enzymes of IMP metabolism: Transformation- and proliferation-linked alterations in gene expression. *Adv. Enzyme Regul.* **14,** 3–24.
11. Collart, F.R. & Huberman, E. (1988) Cloning and sequence analysis of the human and Chinese hamster inosine-5′-monophosphate dehydrogenase cDNAs. *J. Biol. Chem.* **263,** 15769–15772.
12. Collart, F.R., Chubb, C.B., Mirkin, B.L. & Huberman, E. (1992) Increased inosine-5′-phosphate dehydrogenase gene expression in solid tumor tissues and tumor cell lines. *Cancer Res.* **52,** 5826–5828.
13. Natsumeda, Y., Ohno, S., Kawasaki, H., Konno, Y., Weber, G. & Suzuki, K. (1990) Two distinct cDNAs for human IMP dehydrogenase. *J. Biol. Chem.* **265,** 5292–5295.
14. Carr, S.F., Papp, E., Wu, J.C. & Natsumeda, Y. (1993) Characterization of human type I and type II IMP dehydrogenases. *J. Biol. Chem.* **268,** 27285–27290.
15. Konno, Y. Natsumeda, Y., Nagai, M., Yamaji, Y., Ohno, S., Suzuki, K. & Weber, G. (1991) Expression of human IMP dehydrogenase types I and II in *Escherichia coli* and distribution in human normal lymphocytes and leukemic cell lines. *J. Biol. Chem.* **266,** 506–509.
16. Nagai, M., Natsumeda, Y., Konno, Y., Hoffman, R., Irino, S. & Weber, G. (1991) Selective up-regulation of type II inosine 5′-monophosphate dehydrogenase messenger RNA in human leukemias. *Cancer Res.* **51,** 3886–3890.
17. Gebeyehu, G., Marquez, V.E., Kelly, J.A., Cooney, D.A., Jayaram, H.N. & Johns, D.G. (1983) Synthesis of thiazole-4-carboxamide adenine dinucleotide. A powerful inhibitor of IMP dehydrogenase. *J. Med. Chem.* **25,** 922–925.
18. Boritzki, T.J., Berry, D.A., Besserer, J.A., Cook, P.D., Fry, D.W., Leopold, W.R. & Jackson, R.C. (1985) Biochemical and antitumor activity of tiazofurin and its selenium analogue. *Biochem. Pharmacol.* **34,** 1109–1114.
19. Robins, R.K., Srivastava, P.C., Narayanan, V.L., Plowman, J. & Paull, K.D. (1982) 2-β-D-Ribofuranosylthiazole-4-carboxamide, a novel potential antitumor agent for lung tumors and metastases. *J. Med. Chem.* **25,** 107–108.
20. Trump, D.L., Tutsch, K.D., Koeller, J.M. & Tormey, D.C. (1985) Phase I clinical study with pharmacokinetic analysis of 2-β-D-ribofuranosylthiazole-4-carboxamide (NSC 286193)

administered as a five-day infusion. *Cancer Res.* **45,** 2853–2858.

21. *Blood* (1991) **78,** No. 3, cover page.
22. Goldstein, B.M., Leary, J.F., Farley, B.A., Marquez, V.E., Levy, P.C. & Rowley, P.T. (1991) Induction of HL60 cell differentiation by tiazofurin and its analogues: characterization and efficacy. *Blood* **78,** 593–598.
23. Kharabanda, S.M., Sherman, M.L. & Kufe, D.W. (1990) Effects of tiazofurin on guanosine nucleotide binding regulatory proteins in HL-60 cells. *Blood* **75,** 583–588.
24. Weber, G. (1989) Critical issues in chemotherapy with tiazofurin. *Adv. Enzyme Regul.* **29,** 75–95.
25. Jayaram, H.N., Lapis, E., Tricot, G., Kneebone, P., Paulik, E., Zhen, W., Engeler, G.P., Hoffman, R. & Weber, G. (1992) Clinical pharmacokinetic study of tiazofurin administered as 1-hour infusion. *Int. J. Cancer* **51,** 182–188.
26. Prajda, N., Hata, Y., Abonyi, M., Singhal, R.L. & Weber, G. (1993) Sequential impact of tiazofurin and ribavirin on the enzymic program of the bone marrow. *Cancer Res.* **53,** 5982–5986.
27. Olah, E., Natsumeda, Y., Ikegami, T., Kote, Z., Horanyi, M., Szelenyi, J., Paulik, E., Kremmer, T., Hollan, S.R., Sugar, J. & Weber, G. (1988) Induction of erythroid differentiation and modulation of gene expression by tiazofurin in K-562 leukemia cells. *Proc. Natl. Acad. Sci. U.S.A.* **85,** 6533–6537.
28. Weber, G., Nagai, M. & Natsumeda, Y. (1991) Tiazofurin down-regulates expression of c-Ki *-ras* oncogene in a leukemic patient. *Cancer Commun.* **3,** 61–66.
29. Weber, G., Nagai, M. & Natsumeda, Y. (1991) Down-regulation in c-Ki-ras and c-myc expression in tiazofurin-related leukemic patients. *Proc. Amer. Assn. Cancer Res.* **32,** Abst. # 1717.
30. Friedland, A., Conelly, M.C. & Robbins, T.J. (1986) Tiazofurin metabolism in human limphoblastoid cells: Evidence for phosphorylation by adenosine kinase and 5'-nucleotidase. *Cancer Res.* **46,** 532–537.
31. Cooney, D.A., Jayaram, H.N., Gebeyehu, G., Betts, C.R., Kelly, J.A., Marquez, V.E. & Johns, D.G. (1982) The conversion of 2-β-D-ribofuranosylthiazole-4-carboxamide to an analogue of NAD with potent IMP dehydrogenase-inhibitory properties. *Biochem. Pharmacol.* **31,** 2133–2136.
32. Gebeyehu, G., Marquez, V.E., Van Cott, A., Cooney, D.A., Kelley, J.A., Jayaram, H.N., Ahluwalia, G.S., Dion, R.L., Wilson, Y.A. & Johns, D.G. (1985) Ribavirin, tiazofurin, and selenazofurin: Mononucleotides and nicotinamide adenine dinucleotide analogues. Synthesis, structure, and interactions with IMP dehydrogenase. *J. Med. Chem.* **28,** 99-105.
33. Berger, N.A., Berger, S.A., Catino, D.M., Petzold, S.J. & Robins, R.K. (1985) Modulation of nicotinamide adenine dinucleotide and poly (ADP-ribose) metabolism by synthetic "C" nucleoside analogs, tiazofurin and selenazofurin. *J. Clin. Invest.* **75,** 702–709.
34. Jayaram, H.N. (1985) Biochemical mechanism of resistance to tiazofurin. *Adv. Enzyme Regul.* **24,** 67-89.
35. Ahluwalia, G.S., Cooney, D.A., Marquez, V.E., Jayaram, H.N. & Johns, D.G. (1986) Studies on the mechanism of action of tiazofurin (2-β-D-ribofuranozylthiazole-4-carboxamide). VI. Biochemical and pharmacological studies on the degradation of thiazole-4-carboxamide adenine dinucleotide (TAD). *Biochem. Pharmacol.* **35,** 3783-3790.
36. Marquez, V.E., Tseng, C.K.H., Gebeyehu, G., Cooney, D.A., Ahluwalia, G.S., Kelley, J.A., Dalal, M., Fuller, R.W., Wilson, Y.A. & Johns, D.G. (1986) Thiazole-4-carboxamide adenine dinucleotide (TAD). Analogues stable to phosphodiesterase hydrolysis. *J. Med. Chem.* **29,** 1726–1731.
37. Srivastava, P.C. & Robins, R.K. (1983) Synthesis and antitumor activity of 2-β-D-ribofuranosylselenazole-4-carboxamide and related derivatives. *J. Med. Chem.* **26,** 445–448.
38. Franchetti, P., Cristalli, G., Griffantini, M., Cappellacci, L., Sauro, V. & Nocentini, G. (1990) Synthesis and antitumor activity of 2-β-D-ribofuranosyloxazole-4-carboxamide. *J. Med. Chem.* **33,** 2849–2852.
39. Goldstein, B.M., Mao, D.T. & Marquez, V.E. (1988) Ara-tiazofurin: Conservation of structural features in an unusual thiazole nucleoside. *J. Med. Chem.* **31,** 1026–1031.
40. Dishington, A.P., Humber, D.C. & Stoodley, R.J. (1993) C-Nucleosides. Part 2. Preparation of 2-[(1*R*,2*S*,3*R*,4*R*)-2,3-dihydroxy-4(hydroxy methyl)cyclopentyl]-thiazole-4-carboxamide (carbocyclic tiazofurin) and its antipode. *J. Chem. Soc. Perkin Trans.* I, 57–60.
41. Hennen, W.J. & Robins, R.K. (1985) Synthesis of 5-β-D-ribofuranosyl-1,2,4-oxadiazole-4-carboxamide. *J. Heterocycl. Chem.* **22,** 1747–1748.
42. Khorshidi, H.S. & Rodrigez, A.C. (1994) Precursors for the synthesis of substituted 1,3,4-thiadiazole C-nucleosides, analogues of tiazofurin and related compounds. *Nucleosides & Nucleotides,* **13,** 1809–1817.
43. Bergstrom, D.E. & Zhang, P. (1991) An efficient route to C-4-linked imidazole nucleosides:

Synthesis of 2-carbamoyl-4-(2'-deoxy-β-D-ribofuranosyl) imidazole. *Tetrahedron Lett.* **32,** 6485–6487.

44. Reiley, T.A., Hennen, W.J., Kent Dalley, N., Wilson, B.E., Robins, R.K. & Larson, S.B. (1987) Synthesis of 2-(β-D-ribofuranosyl)pyrimidines, a new class of C-nucleosides. *J. Heterocycl. Chem.* **24,** 955–964.

45. Matsuda, A., Minakawa, N., Sasaki, T. & Ueda, T. (1988) The design, synthesis and antileukemic activity of 5-alkynyl-1-β-D-ribofuranosyl-imidazole-4-carboxamides. *Chem. Pharm. Bull.* **36,** 2730–2733.

46. Balzarini, J., Karlsson, A., Wang, L., Bohman, C., Horska, K., Votruba, I., Fridland, A., Van Aerschot, A., Herdewijn, P. & De Clercq, E. (1993) Eicar (5-ethynyl-1-β-D-ribofuranosyl)-imidazole-4-carboxamide. *J. Biol. Chem.* **268,** 24591–24598.

47. Krohn, K., Heins, H. & Wielckens, K. (1992) Synthesis and cytotoxic activity of C-glycosidic nicotinamide riboside analogues. *J. Med. Chem.* **35,** 511–517.

48. Jayaram, H.N., Gharehbaghi, K., Jayaram, N.H., Rieser, J., Krohn, K. & Paull, K.D. (1992) Cytotoxicity of a new IMP dehydrogenase inhibitor, benzamide riboside, to human myelogenous leukemia K562 cells. *Biochem. Biophys. Res. Commun.* **186,** 1600–1606.

49. Gharehbaghi, K., Paull, K.D., Kelly, J.A., Barchi, J.J., Jr., Marquez, V.E., Cooney, D.A., Monks, A., Scudiero, D., Krohn, K. & Jayaram, H.N. (1994) Cytotoxicity and characterization of an active metabolite of benzamide riboside, a novel inhibitor of IMP dehydrogenase. *Int. J. Cancer* **56,** 892–899.

50. Anderson, W.K., Lee, J., Swann, R.T. & Boehm, T.L. (1991) The design, synthesis, and study of mycophenolic acid analogues as potential inhibitors of inosine monophosphate dehydrogenase; in *Advances in New Drug Development* (Kim, B.-K., Lee, E.B., Kim, C.-H. & Han, Y.N., eds.) pp. 8–17, The Pharmaceutical Society of Korea, Seoul.

51. Kabat, M.M., Pankiewicz, K.W. & Watanabe, K.A. (1987) Synthesis of 5-β-D-ribofuranosylnicotinamide and its *N*-methyl derivative. *J. Med. Chem.* **30,** 924–927.

52. Pankiewicz, K.W., Sochacka, E., Kabat, M.M., Ciszewski, L.A. & Watanabe, K.A. (1988) Efficient synthesis of 5-β-D-ribofuranosylnicotinamide and its α-anomer. *J. Org. Chem.* **53,** 3473–3479.

53. Kabat, M.M., Pankiewicz, K.W., Sochacka, E. & Watanabe, K.A. (1988) Synthesis of 6-(β-D-ribofuranosyl)picolinamide. A novel nucleoside from D-ribonolactone. *Chem. Pharm. Bull.* **36,** 634–640.

54. Joos, P.E., Esmans, E.L., Dommisse, R.A., Van Dongen, W., Lepoivre, J.A., Alderweireldt, F.C., Balzarini, J. & De Clercq, E. (1991) Synthesis and biological evaluation of carbamoyl-2-β-D-ribofuranosylpyridine. *Nucleosides & Nucleotides* **10,** 883–894.

55. Pankiewicz, K.W., Zeidler, J., Ciszewski, L.A., Bell, J.E., Goldstein, B.M., Jayaram, H.N. & Watanabe, K.A. (1993) Synthesis of isosteric analogues of nicotinamide adenine dinucleotide containing C-nucleotide of nicotinamide and picolinamide. *J. Med. Chem.* **36,** 1855–1859.

56. Goldstein, B.M., Li, H., Jones, J.P., Bell, J.E., Zaidler, J., Pankiewicz, K.W. & Watanabe, K.A. (1994) C-NAD: A potent and specific inhibitor of alcohol dehydrogenase. *J. Med. Chem.* **37,** 392–399.

57. Li, H., Hallows, W.H., Punzi, J.S., Pankiewicz, K.W., Watanabe, K.A. & Goldstein, B.M. (1994) Crystallographic studies of isosteric NAD analogues bound to alcohol dehydrogenase; Specificity and substrate binding in two ternary complexes. *Biochemistry* **33,** 11734–11744.

58. Li, H., Halows, W.H., Punzi, J.S., Marquez, V.E., Carrell, H.L., Pankiewicz, K.W., Watanabe, K.A. & Goldstein, B.M. (1994) Crystallographic studies of two alcohol dehydrogenase-bound analogues of thiazole-4-carboxamide adenine dinucleotide (TAD), the active anabolite of the antitumor agent tiazofurin. *Biochemistry* **33,** 23–32.

59. Eklund, H. & Branden, C.I. (1987) Crystal structure, coenzyme conformations, and protein interactions; in *Pyridine Nucleotide Coenzymes* (Dolphin, D., Avramovic, O. & Poulson, R., eds.) pp. 74–85, Wiley, New York.

60. Eklund, H. & Branden, C.I. (1987) in *Biological Macromolecules and Assemblies.* Vol. 3: Active Sites of Enzymes. (Jurnak, F.A. & McPherson, A., eds.) Chapt. 2, John Wiley and Sons, New York.

61. Zatorski, A., Goldstein, B.M., Colby, T.D., Jones, J.P. & Pankiewicz, K.W. (1995) Potent inhibitors of human inosine monophosphate dehydrogenase type II; Fluorine-substituted analogues of thiazole-4-carboxamide adenine dinucleotide. *J. Med. Chem.* **38,** 1098–1105.

62. Bergstrom, D.E. & Swartling, D.J. (1988) Fluorine substituted analogs of nucleic acid components; in *Fluorine Containing Molecules, Structure, Reactivity, Synthesis, and Applications* (Liebman, J.F., Greenberg, A. & Dolbier, W.R., Jr., eds.) pp. 259–308, VCH Publishers Inc., New York.

63. Uesugi, S., Miki, M., Ikehara, M., Iwahashi, Y. & Kyogoku, A. (1979) A linear relationship between electronegativity of 2'-substituents and conformation of adenine nucleosides. *Tetrahedron Lett.* 4073–4076.

64. Pankiewicz, K.W., Krzeminski, J. & Watanabe, K.A. (1992) Synthesis of 2'-β-fluoro- and 3'-α-fluoro-substituted guanine nucleosides. Effects of sugar conformational shifts on nucleophilic displacement of the 2'-hydroxy and 3'-hydroxy group with DAST. *J. Org. Chem.* **57**, 7315–7321.

65. Zatorski, A., Lipka, P., Mollova, N., Schram, K.H., Goldstein, B.M., Watanabe, K.A. & Pankiewicz, K.W. (1993) Synthesis of thiazole-4-carboxamide adenine difluoromethylenediphosphonates substituted with fluorine at C2' of the adenosine. *Carbohydr. Res.* **249**, 95–108.

66. Goldstein, B.M., Bell, J.E. & Marquez, V.E. (1990) Dehydrogenase binding by tiazofurin anabolites. *J. Med. Chem.* **33**, 1123–1127.

67. Veres, Z., Szablocks, A., Szinai, I., Denes, G., Kajtar-Peredy, M. & Otvos, L. (1985) 5-Substituted-2,2'-anhydrouridines: Potent inhibitors of uridine phosphorylase. *Biochem. Pharmacol.* **34**, 1737–1740.

68. Westheimer, F.H. (1987) Mechanism of action of the pyridine nucleotites; in *The Pyridine Coenzymes* (Dolphin, D., Avramovic, O. & Poulson, R., eds.) Chapter 9, Wiley, New York.

69. Oppenheimer, N.J., Marschner, T.M., Malver, O. & Kam, B.L. (1986) Stereochemical aspects of coenzyme dehydrogenase interactions; in *Mechanism of Enzymatic Reactions. Stereochemistry* (Frey, P.A., ed.) pp. 15–28, Elsevier Science Publishing Co., New York.

70. Pankiewicz, K.W., Ciszewski, L.A. & Ptak, A.T. (1991) Synthesis of 2,2'-anhydro- 2-hydroxy- and 6,2'-anhydro-6-hydroxy-1-β-D-arabinofuranosylnicotinamide as conformationally restricted nicotinamide nucleoside analogues *Nucleosides & Nucleotides* **10**, 1333–1344.

71. Lipka, P., Zatorski, A., Watanabe, K.A. & Pankiewicz, K.W. (1996) Synthesis of methylene -bridged analogues of nicotinamide riboside, nicotinamide mononucleotide, and nicotinamide adenine dinucleotide. *Nucleosides & Nucleotides* **16**, in press.

72. Pankiewicz, K.W., Zatorski, A. & Watanabe, K.A. (1994) The chemistry of NAD analogues. *Nucleic Acids Res. Symp. Ser.* **31**, 139–142.