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Methylenebis(sulfonamide) linked nicotinamide adenine dinucleotide analogue as an inosine monophosphate dehydrogenase inhibitor

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Abstract—A methylenebis(sulfonamide) linked NAD analogue has been designed to circumvent the metabolically unstable, ionic nature of the natural pyrophosphate linkage. This NAD analogue is assembled through two Mitsunobu reactions of a methylenebis(sulfonamide) linker with two protected nucleosides. A 2,4-dimethoxybenzyl group is used as a sulfonamide protective group, which allows facile removal under mildly acidic conditions. This NAD analogue inhibits IMPDH at low micromolar concentration.

Nicotinamide adenine dinucleotide (NAD) contains a pyrophosphate linkage which is present in numerous biologically important molecules. In addition to NAD's key role in redox reactions in cells, it has recently been found to be involved in various biological processes including post-translational protein modification and signal transductions.[1] The pyrophosphate linkage is also a crucial component in the activated form of glycosyl donors used by numerous glycosyl transferases that participate in oligosaccharide and glycoprotein syntheses. Furthermore, exogenous nucleosides, such as tiazofurin and benzamide riboside, can be metabolically activated and converted into the corresponding NAD analogues such as TAD (**1**, Fig. 1) through the formation of a pyrophosphate linkage.[2,3] These NAD analogues are potent inhibitors of IMP-dehydrogenase (IMPDH), which catalyzes the rate-limiting step in the de novo synthesis of guanine nucleotides. However, such pyrophosphate linked molecules are readily cleaved by cellular enzymes such as phosphodiesterases and their ionic nature prevents them from penetrating the cell membrane. Therefore, there is a growing interest in design and synthesis of metabolically stable and preferably neutral mimics of the pyrophosphate moiety of NAD and other biologically important pyrophosphates.[4–7]

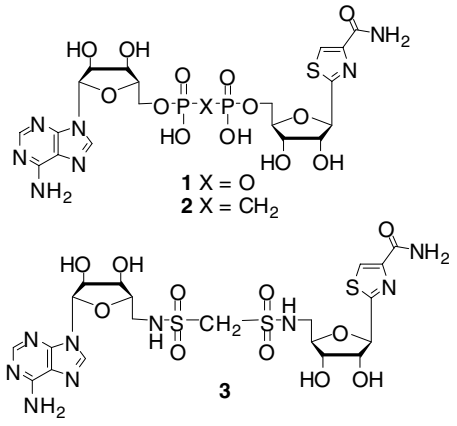

Figure 1. TAD and analogues.

Our laboratory has been actively pursuing NAD analogues as potent inhibitors of IMPDH, which in recent years has emerged as a major therapeutic target for the design of immunosuppressant, anticancer and antiviral agents.[8]

Keywords: Inosine monophosphate dehydrogenase; IMPDH; NAD analogue; Pyrophosphate mimics; Methylenebis(sulfonamide).
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We and others have developed pyrophosphate mimics such as methylenebis(phosphonate) analogues of TAD (**2**),[9,10] other bis(phosphonate)s,[10–12] and phosphonophosphates[13] Herein we report our design and synthesis of NAD analogue **3** in which two nucleoside moieties are connected via a methylenebis(sulfonamide) linker (Fig. 1). We expect that the weakly acidic bis(sulfonamide) functionality will not ionize under physiological conditions. At the same time, the partial negative charge delocalized onto the oxygen atoms would sufficiently mimic the negative charges present in the naturally occurring pyrophosphate linkage. Furthermore, with tetrahedral sulfur atoms, the proposed methylenebis(sulfonamide) linker is expected to closely resemble the geometry of the methylenebis(phosphonate) linker which mimics the pyrophosphate moiety well.

NAD analogue **3** was assembled by two sequential Mitsunobu reactions (Scheme 2) between properly protected nucleosides **4** and **5**,[14] and the sulfonamide moiety, followed by removal of the protective groups (Fig. 2). The key bis(sulfonamide) intermediate **7** (Scheme 1) was readily prepared by reaction of bis(sulfonyl) chloride **6**[15] with benzylamine in high yield. The first Mitsunobu reaction incorporated an isopropylidene protected adenosine **4**, while the second introduced an isopropylidene protected tiazofurin **5** to give protected bis(sulfonamide) **11** albeit in low yields (Scheme 2). With two nucleosides in place, hydrogenolysis was attempted to remove the benzyl protective groups present in **11**. Unfortunately no cleavage of benzyl groups was observed according to ^{1}H NMR. This finding was not unexpected considering the fact that cleavage of sulfonamide benzyl groups has been previously demonstrated to be difficult.[16,17]

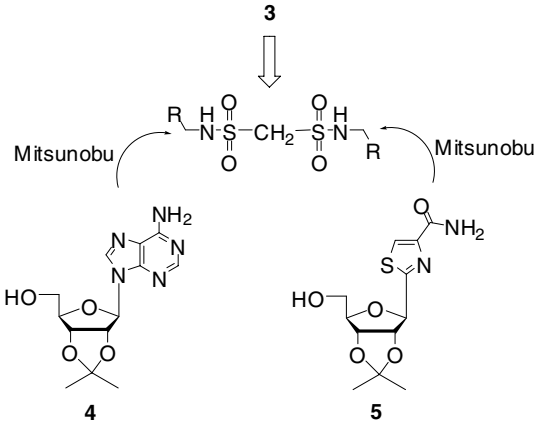
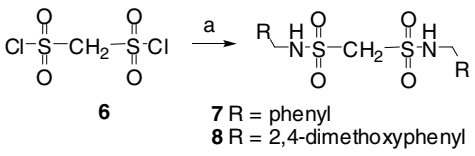
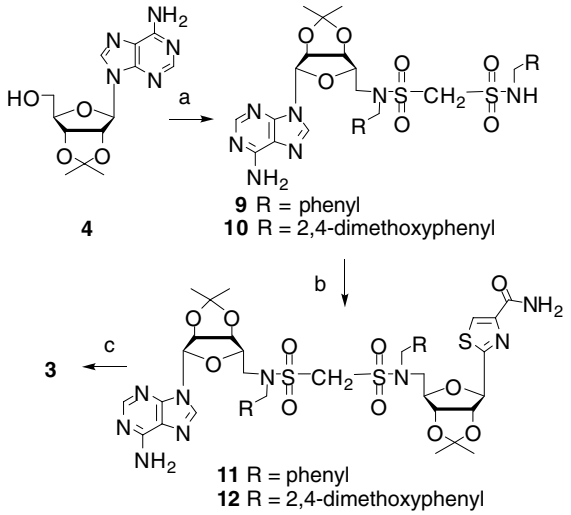


Figure 2. Retrosynthetic scheme of **3**.



Scheme 1. Reagents and conditions: (a) benzylamine or 2,4-dimethoxybenzylamine, THF, 0 °C, 82% for **7** and 94% for **8**.



Scheme 2. Reagents and conditions: (a) DIAD, PPh_3, **7** or **8**, THF, 0 °C to rt, 20% for **9** and 53% for **10**; (b) DIAD, PPh_3, **5**, THF, 0 °C to rt, 15% for **11** and 27% for **12**; (c) 40% (v/v) TFA, Et_3SiH, CH_2Cl_2, and then TFA, H_2O, 54% starting from **12**.

To circumvent this problem, we designed a new bis(sulfonamide) intermediate **8** (Scheme 1) which contains 2,4-dimethoxybenzyl protective groups. The 2,4-dialkoxybenzyl moiety, which can be cleaved under mild acidic conditions, has found broad applications in solid-phase organic synthesis. For instance, acid sensitive methoxy benzaldehyde linker (AMEBA) has been used for the solid-phase syntheses of secondary amide, sulfonamide, and carbamate derivatives.[18] Recently fluorous-tagged[19] and ionic liquid[20] version of AMEBA have been developed. We expected that substitution of benzyl with 2,4-dimethoxybenzyl would allow facile cleavage under mild acidic conditions after the assembly of a protected dinucleotide analogue.[17,21]

Thus new bis(sulfonamide) intermediate **8** was prepared in excellent yield under conditions similar to those described above for **7**. It was subjected to a sequence of two Mitsunobu reactions to give **12** (Scheme 2). At least three equivalents of acidic components involved in the Mitsunobu reactions, for example, **8** and **10**, should be used. Slow addition of these acidic components was also found to be crucial. A smooth removal of 2,4-dimethoxybenzyl groups from **12** was accomplished as expected under mild conditions. Subsequent one-pot acidic hydrolysis of isopropylidene protective groups afforded the desired NAD analogue **3**.[22]

The desired methylenebis(sulfonamide) NAD analogue **3** was evaluated against isoforms of human IMPDH.[13] It showed IC_{50} values of 23.6 and 18.8 μM against the type I and type II enzymes, respectively. However, this NAD analogue did not show anti-proliferation activity against the K562 cell line (IC_{50} > 100 μM).

Analysis of the crystal structures of type II IMPDH with two known inhibitors C2-MAD (**13**, Fig. 3),[11] SAD (**14**), and NAD (PDB entries 1NF7, 1B3O, and 1NFB)[23] suggests that in each case, one of the phosphate oxygen

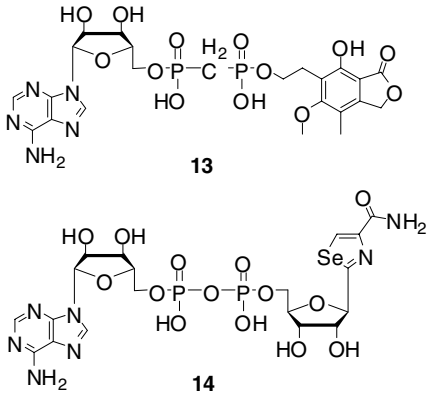


Figure 3. Structures of C2-MAD (**13**) and SAD (**14**).

atoms must be protonated, although the specific interactions are different in each case. For example, in C2-MAD,[11] the mycophenolic phosphate has one oxygen 3.0 Å away from water 73. Because water 73 is donating one of its hydrogens to Gln 441 and its other hydrogen is shared between the carboxylate oxygens of Asp 470, the phosphate oxygen is likely protonated. In the NAD cocrystal structure, the hydroxyl of Ser 275 is within hydrogen bonding distance of the Gln 277 backbone carbonyl and an oxygen of the adenosine phosphate. Finally, in the structure of SAD (Fig. 4), a close analogue of TAD (**1**), the 3′-hydroxyl of the selenazofurin sugar is within hydrogen bonding distance of both the Asp 274 backbone carbonyl and one of the selenazofurin phosphate oxygens.

The requisite protonation of one phosphate oxygen, as indicated by our structural analysis, might explain the lower potency of compound **3**, in which the methylenebis(sulfonamide) linkage cannot allow protonation on oxygen atoms. This lack of protonation would prevent the methylenebis(sulfonamide) linker form engaging in hydrogen bonding interactions, which are crucial for inhibitory activities. The lower activity of the bis(sulfonamide) analogue present here also suggests that activity

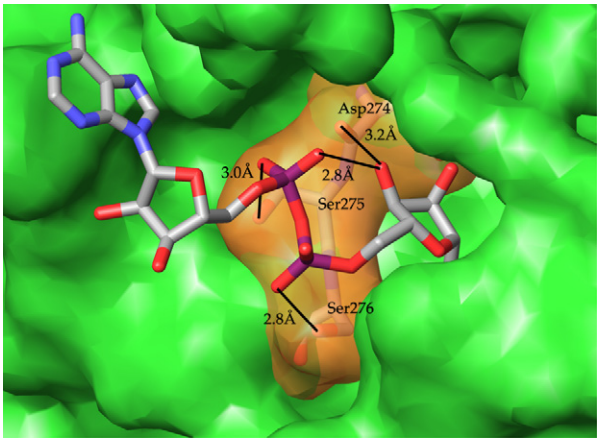


Figure 4. Linker interactions of **14** with IMPDH (PDB entry 1B3O). The surface of residues 274–276 is shown in orange, with the remainder of the protein surface in green.

may depend on the linker being adaptable to different hydrogen bonding situations as the protein structure changes during the catalytic cycle,[24] although the interactions are not consistent among the X-ray structures. Currently we are exploring alternative linkers which have an enhanced ionic character and contain structural features that can interact with the amino acid residues surrounding the linker region.

In summary, we have designed a methylenebis(sulfonamide) linked NAD analogue that shows inhibitory activity against human type I and type II IMPDH. We have devised a convergent synthesis that involves a sequence of two Mitsunobu reactions and facile removal of 2,4-methoxybenzyl protective groups. Given the mild conditions utilized in our synthesis, it is expected that our approach will find broad application in the synthesis of methylenebis(sulfonamide) as a mimic of pyrophosphate linkages which are present in various biologically important molecules.

Acknowledgements

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22. Methylenebis(sulfonamide) linked NAD analogue **3** was prepared from **12** according to the following procedures: A solution of **12** (119.7 mg, 0.114 mmol) in dry CH_2Cl_2 (5 mL), TFA (4 mL), and Et_3SiH (1 mL) was stirred at rt for 7 h. After concentration, the residue was dissolved in TFA (4 mL) and water (1 mL), and the solution was allowed to stir at rt overnight. After concentration and co-evaporation with MeOH several times, the residue was then dissolved in MeOH (ca. 5 mL) and the solid formed, after overnight, was filtered to give compound **3** (40.9 mg, 54%) as a white solid. ^{1}H NMR (DMSO, 600 MHz) δ8.65 (dd, J = 7.5, 4.5 Hz, 1H, exchangeable with D_2O), 8.30 (s, 1H), 8.22 (s, 1H), 8.17 (s, 1H), 7.65 (br s, 2H, exchangeable with D_2O), 7.54 (br s, 1H, exchangeable with D_2O), 7.36 (br s, 2H, exchangeable with D_2O), 5.84 (d, J = 7.2 Hz, 1H), 5.48 (d, J = 6.6 Hz, 1H, exchangeable with D_2O), 5.45 (d, J = 5.4 Hz, 1H, exchangeable with D_2O), 5.28 (d, J = 3.6 Hz, 1H, exchangeable with D_2O), 5.16 (d, J = 6.0 Hz, 1H, exchangeable with D_2O), 5.00–4.92 (m, 3H), 4.69 (dd, J = 11.4, 6.0 Hz, 1H), 4.16–4.04 (m, 3H), 3.94 (dd, J = 10.8, 5.4 Hz, 1H), 3.85 (dd, J = 10.5, 5.6 Hz, 1H), 3.40–3.31 (m, 3H), 3.26–3.19 (m, 1H). ^{13}C NMR (DMSO, 150 MHz) δ 171.3, 162.2, 156.2, 152.5, 150.4, 148.7, 140.4, 124.4, 119.5, 88.3, 84.0, 82.7, 82.1, 76.4, 72.4, 72.0, 71.2, 67.1, 45.4, 45.0. HRMS calcd for $C_{20}H_{28}N_9O_{11}S_3$, 666.1064 $(M+H)^+$; found, 666.1065.
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