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Biomimetic systems involving sequential redox reactions in glycolysis – the sulfur effect†

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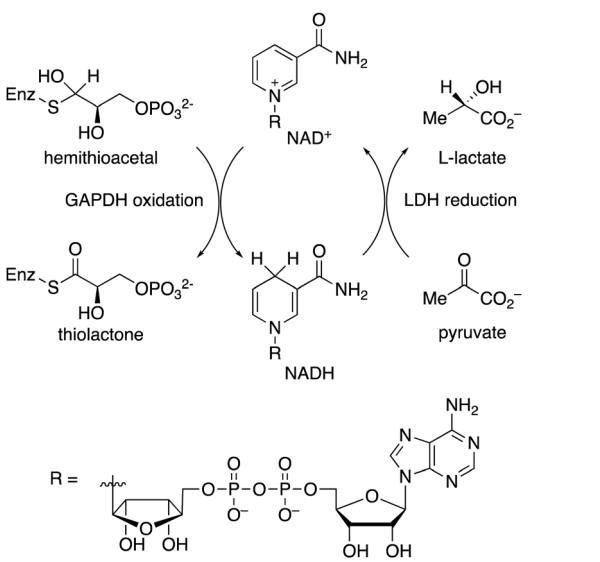
Magnesium hemithioacetates were used as model cysteine compounds to mimic natural hemithioacetals, and their biomimetic oxidation reactions using a model NAD$^+$ compound were investigated. Cyclic hemithioacetate was found to be the best substrate for the reaction with the model NAD$^+$ compound, which gave the corresponding NADH analog in excellent yield.

Coenzyme NAD$^+$ and its reduced form (NADH) function as redox agents in a number of biological reactions including the glycolysis process observed during carbohydrate metabolism.[1] NAD$^+$ oxidizes glyceraldehyde-3-phosphate to bisphosphoglyceric acid in D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH), while NADH paired with the oxidation step reduces pyruvate to L-lactate with high enantioselectivity in lactate dehydrogenase (LDH) during anaerobic glycolysis (Scheme 1). Consequently, there have been a large number of artificial NADH model compounds reported for LDH-type asymmetric reduction reactions,[2] but only a few examples have been studied in the GAPDH-type biomimetic oxidation reaction.[3] We have previously reported that regioselective hydrogen transfer from a 1,1-diolate, a model substrate mimicking the biological system, proceeds very efficiently to form a series of model NAD$^+$ compounds during the GAPDH-type reaction without the use of a transition metal catalyst.[3a] However, natural biological oxidation during glycolysis is known to proceed *via* a hemithioacetal intermediate produced *via* the addition of a cysteine residue to an aldehyde substrate in GAPDH. To develop a process similar to that found in biological systems, we have designed an active hemithioacetal derivative representing a new class of model compounds generated from D-glyceraldehyde-3-phosphate and the cysteine residue in GAPDH. In this paper, we report the biomimetic oxidation of a model NAD$^+$ compound using a series of hemithioacetal derivatives *via* a biomimetic system involving sequential redox reactions.

An active acyclic hemithioacetal derivative **2** was prepared *in situ* by the reaction of the magnesium thiolate derived from ethanethiol (**1**) and acetaldehyde in THF (Table 1). We initially investigated the biomimetic oxidation reaction using compound **2** and *N*-benzyl pyridinium salt **3a**, a model NAD$^+$ compound. A regioselective hydrogen transfer reaction occurred from **2** to **3a** to exclusively give 1,4-dihydropyridine **4a**[3a,4] in up to 23% yield (entry 1). Efforts to increase the product yield by the addition of a polar aprotic co-solvent such as DMF or the use of an excess amount of **2** were unsuccessful (entries 2 and 3). Performing the reaction at higher temperature was inefficient (entry 4) and indicated acyclic hemithioacetal derivative **2** was not very stable upon heating. Although our



Scheme 1 GAPDH and LDH holoenzymatic redox during anaerobic glycolysis.

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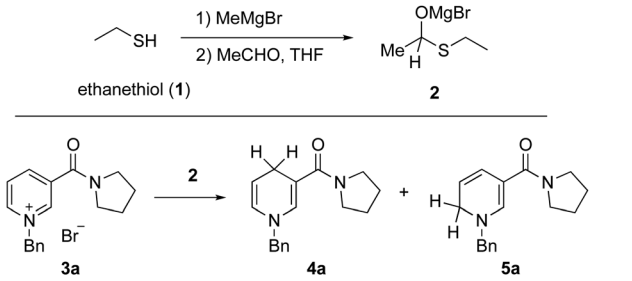
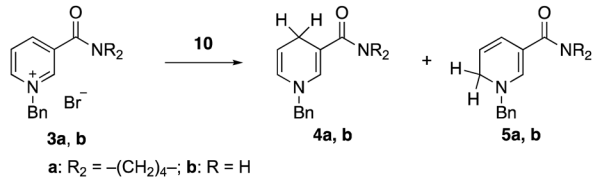
Table 1 Biomimetic oxidation using acyclic hemithioasetal derivative **2**



Entry	**2** (equiv.)	Temp. (°C)	Solvents	**4a** (%)[a]	Ratio (**4a** : **5a**)[b]
1	1	50	THF	23	>99 : 1
2	1	50	THF/DMF = 5 : 1	25	>99 : 1
3	3	50	THF/DMF = 5 : 1	23	>99 : 1
4	1	90	THF	12	>99 : 1

[a] Isolated yield. [b] Determined by ^{1}H NMR.

Table 2 Biomimetic oxidation using cyclic hemithioacetal derivative **10**



Entry	**10** (equiv.)	**3**	Temp. (°C)	Time (h)	Solvents	**4**[a] (%)	Ratio (**4** : **5**)[b]
1	5	**3a**	90	1	THF	61	>99 : 1
2	2	**3a**	90	1	THF	31	>99 : 1
3	2	**3a**	90	1	THF/DMF = 6 : 1	72	>99 : 1
4	2	**3a**	90	1	THF/DMF = 4 : 1	80	>99 : 1
5	2	**3a**	90	1	THF/HMPA = 6 : 1	6	>99 : 1
6	2	**3a**	90	1	THF/DMSO = 6 : 1	Trace	—
7	5	**3a**	50	1	THF/DMF = 4 : 1	Trace	—
8	5	**3a**	50	3	THF/DMF = 4 : 1	47	>99 : 1
9	5	**3a**	50	6	THF/DMF = 4 : 1	37	>99 : 1
10	2	**3b**	90	1	THF/DMF = 4 : 1	74	>99 : 1
11	2	**3b**	50	3	THF/DMF = 4 : 1	39	>99 : 1

[a] Isolated yield. [b] Determined by ^{1}H NMR.

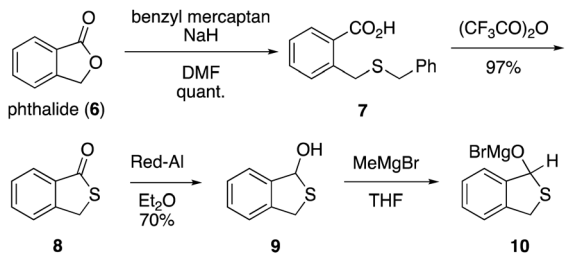
initial attempts at modeling the biooxidation reaction were unsatisfactory, it is still worth noting that none of the 1,6-dihydropyridine isomer **5a** was formed in the reaction upon careful examination of the crude products by ^{1}H NMR spectroscopy.[3a,4c]

Based on our assumption that the thioethyl group is easily removed from **2** at high temperature required for breaking the aromaticity of the pyridinium moiety, we subsequently designed cyclic hemithioacetate derivative **10** as a new class of model substrate for the GAPDH-type oxidation reaction (Scheme 2). The synthesis of **10** is shown in Scheme 2. Phthalide (**6**) was reacted with benzyl mercaptan in DMF to give carboxylic acid **7** in quantitative yield. A subsequent cyclization reaction of **7** in the presence of trifluoroacetic anhydride afforded thiolactone **8** in 97% yield.[5,6] After the reduction of **8** using Red-Al, the resulting cyclic hemithioacetal **9** was treated with methylmagnesium bromide to give hemithioacetal derivative **10**, which was used in the biomimetic oxidation reaction without isolation.

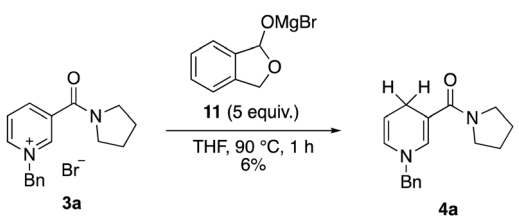
The reaction of model NAD$^+$ compound **3** with 5 equiv. of **10** in THF at 90 °C afforded 1,4-dihydropyridine **4a** in 61% yield (Table 2, entry 1). In this reaction, thiolactone **8** was isolated in 54% yield. The reaction using 2 equiv. of **10** under similar reaction conditions also proceeded to give **4a**, albeit in 31% yield (entry 2). Subsequently, we carried out the reaction in the presence of different amounts of DMF as an aprotic co-solvent and the regioselective hydrogen transfer reaction of **9** took place at 90 °C to give **4a** in 72 and 80% yield, respectively (entries 3 and 4). The reaction was performed using HMPA and DMSO as the reaction cosolvent, but the product yield was low (entries 5 and 6). The biomimetic reaction performed at 50 °C afforded NADH model compound **4a** in trace amounts (entry 7). However, extending the reaction time gave **4a** in 47% yield (entry 8). Furthermore, the reactivity in the biomimetic oxidation did not improve when conducting the reaction for more than 3 h (entry 9). In entries 10 and 11, we performed a biomimetic oxidation using NAD$^+$ model **3b**. As a result, the yield was similar to that when **3a** was used (*vs.* entries 4 and 8). 1,6-Dihydropyridine **5a**, **b** was formed in the reaction, but in trace amounts in all entries. The regioselectivity of NADH models **4** and **5** was improved compared to our previous results of the biomimetic oxidation using 1,1-diolate.[3a] The use of DMF as a co-solvent is useful to improve both the reactivity and the regioselectivity affording the desired a 1,4-dihydropyridine motif as an NADH model.[4c]

The reaction using cyclic hemiacetal derivative **11** was performed in order to compare its reactivity and selectivity with those of compound **10** (Scheme 3).

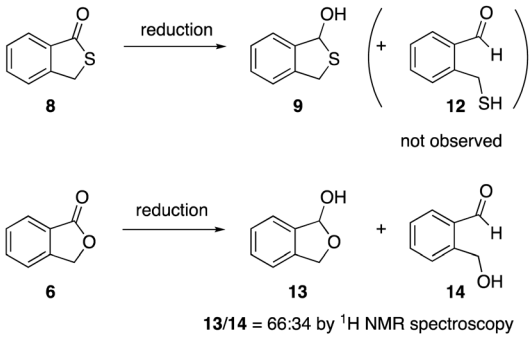
The reaction using model NAD$^+$ compound **3** and **11** performed at 90 °C in THF also gave compound **4a**, but the product yield was low (6%), which was in remarkable contrast to the result obtained using sulfur analog **10** (Table 2, entry 1).



Scheme 2 Synthesis of cyclic hemithioacetal derivative **10**.



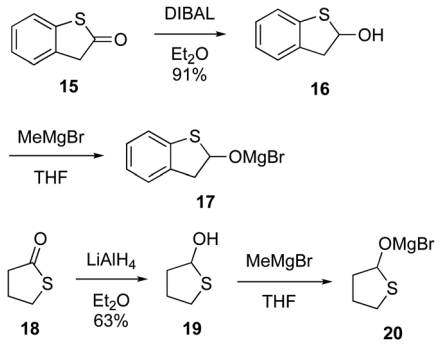
Scheme 3 Biomimetic oxidation using hemiacetal derivative **11**.



Scheme 4 Existence ratio of hemithioacetal **9** and hemiacetal **13**.

The difference observed between the ''oxygen'' and ''sulfur'' reactions was apparently dependent on the abundance of the acetal and thioacetal formed under the reaction conditions (Scheme 4). The ^{1}H NMR spectrum of hemithioacetal **9** showed no significant peak beyond δ 8.0 ppm, indicating that the formation of aldehyde **12** was negligible in its equilibrium state. On the other hand, hemiacetal **13** exists as an equilibrium mixture with aldehyde **14** in a 66 : 34 ratio, as determined by ^{1}H NMR spectroscopy (see ESI†). This sulfur effect favoring the ring-closed form of the hemithioacetal is directly connected to higher efficiency observed in the hydrogen transfer step from **10** compared to **11**. However, there must be a more hidden sulfur effect: this is because the yield of **4a** with reactive hemithioacetate **10** was *ca.* 10 times larger than that observed with **11**, which exceeds the value estimated from their abundances determined using ^{1}H NMR spectroscopy. The superb reactivity exhibited during the hydrogen transfer process from **10** depends on the higher electron-donating effect of sulfur compared to oxygen.[7] This interpretation is also closely related to GADPH oxidation observed in nature, which employs a cysteine residue to activate glyceraldehyde-3-phosphate. A deep and rigid pocket in the enzyme binding site of the substrate may also prevent the entropic loss observed in the system.

Further biomimetic reactions were investigated using isomeric **17** and aliphatic **20**. Thiolactone **15** was prepared from commercially available benzo[*b*]thiophen-2-boronic acid using a literature procedure (Scheme 5).[8] During the reduction of **15** using DIBAL, hemithioacetal **16** was produced as the sole isomer (the ring-opened aldehyde was not observed by ^{1}H NMR spectroscopy). Finally, hemithioacetal derivative **17** was synthesized *in situ* upon reaction with methylmagnesium bromide. Magnesium hemithiacetate **20** was generated from γ-butylothiolactone (**18**) *via* hemithioacetal **19**. The ring-opened aldehyde formed from cyclic hemithioacetal **19** was not observed using ^{1}H NMR spectroscopy, being consistent with the other hemthioacetals, **9** and **16**.

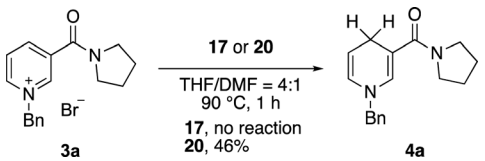


Scheme 5 Synthesis of magnesium hemithioacetate **17** and **20**.

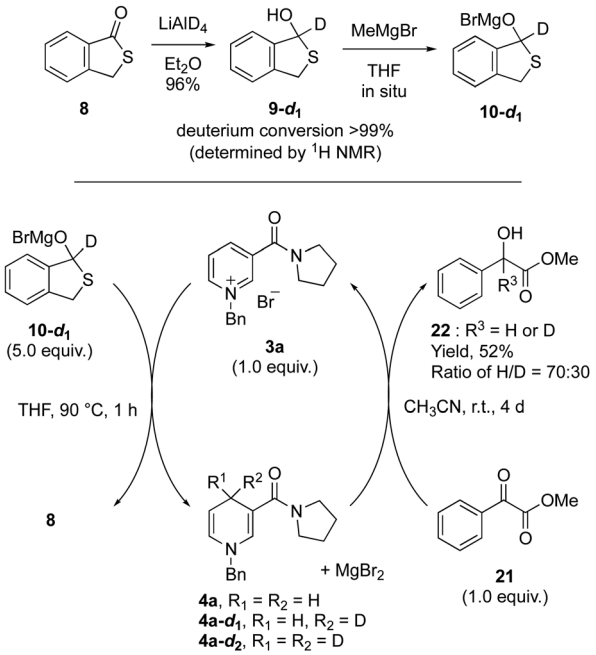
We carried out the biomimetic reaction using magnesium hemithioacetate **17** and **20** (Scheme 6). The reduction using hemithioacetate **17** did not take place under the optimized reaction conditions shown in Table 2. On the other hand, the reaction using hemithioacetate **20** gave 1,4-dihydropyridine **4** in 46% yield. These results indicate that the reactivity of **10**, which has a dihydrobenzo[*c*]thiophenol structure, was remarkably higher than that observed for the isothiochromanol (**17**) and tetrahydrothiophenol (**20**) derivatives in the biomimetic oxidation reaction. Hydrogen, a vital component in the stereoselective biomimetic reduction, acquires the highest reactivity at a benzylic position of **10**, followed by that of aliphatic **20**. The sulfur atom conjugated with the benzene ring deprives the hydrogen atom of its reactivity, as is exemplified in **17**.

We have designed a one-pot sequential biomimetic oxidation and reduction process mediated by an NAD^+/NADH system, which can be used as a mimic of anaerobic glycolysis. In addition, we recognized that a stoichiometric amount of Mg^{2+} is released as an essential activator for the biomimetic reduction of the pyruvate analog when using the synthetic NADH analog (Scheme 7).[3a,9]

The reaction using deuterated cysteine derivative **10-d$_1$** resulted in the formation of **4a** and the C-4 deuterated 1,4-dihydropyridines **4a-d$_1$** and **4a-d$_2$**, which was attributed to the deuterium–hydrogen scrambling catalyzed by **3a**.[3a] After removal of the reaction solvent (THF), a LDH-type reaction using methyl benzoylformate (**21**) in CH_3CN was carried out without any further addition of magnesium salts. After stirring at room temperature for 4 d, the desired product, methyl mandelate (**22**), was obtained and the hydrogen–deuterium ratio was 70 : 30 by ^{1}H NMR spectroscopy. We then investigated the reduction of **21** using the mono-deuterated NADH analog **4a-d$_1$**, which was prepared independently from **3** and sodium dithionite in D_2O in the presence of magnesium perchlorate.[3a,10] Partially deuterated **22** was obtained with an identical H/D ratio of 70 : 30.[11] This value was in good agreement with that obtained in the relay model reaction when considering the ratio of **4a**, **4a-d$_1$**, and **4a-d$_2$** formed in the preceding reaction. These findings clearly show that the preferential deuterium–hydrogen relay



Scheme 6 Biomimetic oxidation using hemithioacetal derivative **17** and **20**.



Scheme 7 Synthesis of deuterated hemithioacetal derivative **10-d$_1$**, and the sequential biomimetic oxidation and reduction system.

transfer reaction from **10-d$_1$** to **22** was mediated by the redox cycle of the model NAD^+/NADH compounds as well as the real coenzyme system during glycolysis.

In summary, we have accomplished a GAPDH-type oxidation reaction using a series of hemithioacetal derivatives in a model NAD^+/NADH system and demonstrated its application in GAPDH- and LDH-type relay model reactions. When we carried out the biomimetic oxidation reaction using cyclic hemithioacetate **10** bearing a five-membered ring, the hydrogen transfer reaction proceeded under mild conditions using a THF/DMF solvent system. These results indicate that the reactivity of **10** in the biomimetic oxidation reaction was better than that observed for compounds **2**, **11**, **17**, and **20**. A further detailed mechanistic study exploring the scope and limitation of the reaction as well as its application to a model catalytic system is now actively underway in our laboratories.

Conflicts of interest

There are no conflicts to declare.

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11 The NADH model compound **4** did not reduce **21** to **22** in the absence of Mg^{2+}. Therefore, the preceding GAPDH-type model reaction must provide an activator such as $MgBr_2$ to the following LDH-type model reaction.

