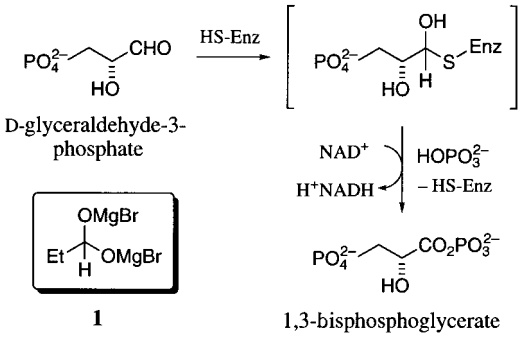
Biomimetic Oxidation of Aldehyde with NAD$^+$ Models: Glycolysis-Type Hydrogen Transfer in an NAD$^+$/NADH Model System**

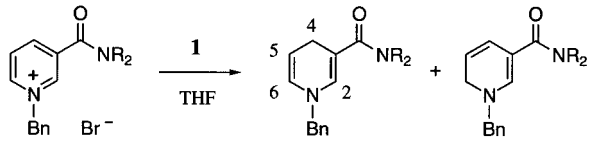
Nobuhiro Kanomata,* Masayuki Suzuki, Mamiko Yoshida, and Tadashi Nakata

Coenzyme NAD$^+$ and its reduced form, NADH, function as redox reagents for biological reactions that are catalyzed by L-lactate and D-glyceraldehyde-3-phosphate dehydrogenases (LDH and GAPDH) in anaerobic glycolysis.[1] A number of synthetic NADH anologues have been reported, including our bridged NADH models[2a] for LDH-type asymmetric reduction,[2, 3] but few have been studied in biomimetic oxidation. Although some NAD$^+$ models oxidize alcohol/alkoxide[4, 5] or formate[6] with formation of the NADH analogues and the corresponding oxidation products, GAPDH oxidation of aldehyde to caboxylate has not been mimicked so far.[7] To realize this unprecedented model reaction, we focused our attention on the aldehyde equivalent **1**,[8] which is an analogue of the hemithioacetal intermediate[1, 9] formed from D-glyceraldehyde-3-phosphate and a cysteine residue of GAPDH (Scheme 1). Here we describe the first GAPDH-analogous oxidation of aldehyde to carboxylate in combination with highly selective 1,4-reduction of NAD$^+$ to NADH model compounds and its application to GAPDH- and LDH-type model transfer reactions.



Scheme 1. GAPDH oxidation and structure of **1**.

The diolate **1** was prepared according to the known procedure.[8] The regioselective hydrogen transfer from **1** proceeds most efficiently with *N*-benzylpyridinium salts (**2**) that contain tertiary amide groups (Table 1; see Table 2 for spectroscopic data for **2**–**4**). The reaction of **2a** with **1** (5 or 10 equiv) was carried out in THF at room temperature for 5 h to give 1,4-dihydronicotinamide **3a**[10] almost exclusively (entries 1 and 2). The reactions at higher temperatures were complete within 1–3 h and led to preferential formation of **3a** and a small amount of 1,6-dihydronicotinamide **4a**[11] (ratio ca. 9:1; entries 3 and 4). Propionic acid, the product of the oxidation of **1**, was isolated from acidic aqueous solution and identified by means of its ^{1}H NMR spectrum. High 1,4-selectivity was also exhibited by the other NAD$^+$ models **2b**, **2c**,[4d] and **2d**,[12] which gave predominantly the desired NADH anologues **3b**, **3c**,[4d] and **3d**[13] together with the corresponding 1,6-isomers **4b**–**d**[11] in a ratio greater than 9:1 (entries 5–7 and 9). The 1,4-selectivity decreased with increasing reaction time (entries 8 and 10); this is probably due to isomerization of **3c**, **d** to **4c**, **d** in the presence of the pyridinium salts **2c**, **d**.[14]



a: $R_2 = -(C_4H_8)-$; **b**: $R_2 = -(C_5H_{10})-$; **c**: $R_2 = -(C_2H_4OC_2H_4)-$; **d**: R = Me

Table 1. Reactions of **2a**–**d** with **1**.[a]

Entry	**2**	T [°C]	t [h]	**3**:**4**[b]	Yield [%][c]
1	**2a**	RT	5	>95:<5	58
2[d]	**2a**	RT	5	>95:<5	87
3	**2a**	50	3	91:9	73
4	**2a**	90	1	91:9	92
5[d]	**2b**	RT	5	93:7	61
6	**2b**	90	1	95:5	90
7	**2c**	90	1	91:9	72
8	**2c**	90	4	84:16	62
9	**2d**	90	1	93:7	69
10	**2d**	90	4	73:27	59

[a] All reactions were carried out in THF under argon with 5 equiv of **1** unless otherwise stated. [b] Ratios determined from the integrals of the ^{1}H NMR spectra. [c] Yields are of crude mixtures of **3** and **4** after extraction. [d] 10 equiv of **1**.

An isotope experiment unequivocally proved that these selectivities are the result of kinetic control: The reaction of **2a** with the deuterated diolate [D$_1$]-**1** (prepared from [D$_2$]formic acid) was carried out in THF at 50°C for 3 h. The 1,4-dihydronicotinamide obtained after column chromatography on aluminum oxide was a mixture of **3a** and its mono- and dideuterated compounds [D$_1$]-**3a** and [D$_2$]-**3a** in a 28:60:12 ratio (Scheme 2). This is ascribed to deuterium/hydrogen exchange by **2a**-catalyzed transhydrogenation.[15] The ^{2}H NMR spectrum of the mixture indicated that the deuterium atom is attached exclusively at the C4 position ($\delta = 3.18$). Therefore, in the model reactions, the reactive hydrogen atom of **1** is transferred to the C4 position of NAD$^+$ model compounds **2a**–**d**. This process is comparable to the direct hydrogen transfer[16] from the hemithioacetal intermediate to coenzyme NAD$^+$ in natural GAPDH oxidation.

[*] Dr. N. Kanomata, M. Suzuki, M. Yoshida, Prof. Dr. T. Nakata
The Institute of Physical and Chemical Research (RIKEN)
Wako-shi, Saitama 351-0198 (Japan)
Fax: (+81) 48-462-4666
E-mail: nobkano@riken.go.jp

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Table 2. Melting points and NMR for **2**–**4**.

2a: White solid; m.p. 172–175 °C; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 1.93$–2.01 (m, 4H), 3.61 (t, $J = 6.6$ Hz, 2H), 3.69 (t, $J = 6.3$ Hz, 2H), 6.44 (s, 2H), 7.34–7.41 (m, 3H), 7.68–7.71 (m, 2H), 8.15 (dd, $J = 7.9$, 5.9 Hz, 1H), 8.60 (d, $J = 7.9$ Hz, 1H), 9.53 (s, 1H), 9.71 (d, $J = 5.9$ Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 24.0$, 26.3, 46.9, 49.6, 64.0, 128.4, 129.5(2C), 129.6(2C), 129.9, 132.6, 136.9, 143.2, 143.8, 145.7, 161.5.

2b: White solid; m.p. 202–204 °C; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 1.51$–1.75 (m, 6H), 3.45 (m, 2H), 3.66 (m, 2H), 6.40 (s, 2H), 7.36–7.42 (m, 3H), 7.66–7.69 (m, 2H), 8.13 (dd, $J = 7.9$, 6.3 Hz, 1H), 8.38 (d, $J = 7.9$ Hz, 1H), 9.27 (s, 1H), 9.74 (d, $J = 6.3$ Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta =$ 23.9, 25.2, 26.5, 43.6, 49.3, 64.4, 128.4, 129.6(2C), 129.8(2C), 130.1, 132.4, 136.7, 142.8, 143.4, 145.6, 162.3.

[D_1]-**3a**: Oil; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.81$ (tt, $J = 6.8$, 3.0 Hz, 4H), 3.16 (br s, 1H), 3.45 (tt, $J = 6.8$, 3.0 Hz, 4H), 4.23 (s, 2H), 4.63 (dd, $J = 8.1$, 3.4 Hz, 1H), 5.74 (dt, $J = 8.1$, 1.5 Hz, 1H), 6.44 (br s, 1H), 7.24 (dd, $J = 7.7$, 1.7 Hz, 2H), 7.27, (tt, $J = 7.7$, 1.7 Hz, 1H), 7.34 (t, $J = 7.7$ Hz, 2H).

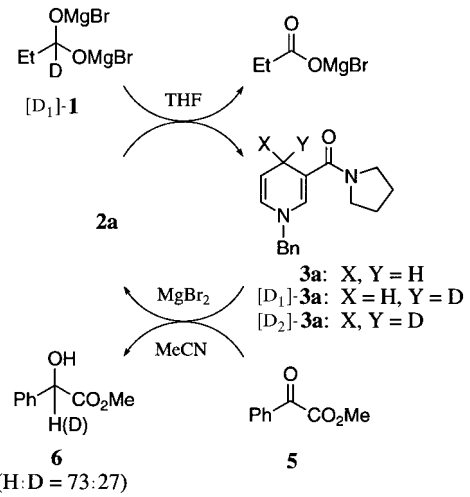
3b: Oil; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.49$ (quint, $J = 5.6$ Hz, 4H), 1.61 (quint, $J = 5.7$ Hz, 2H), 3.14 (dd, $J = 3.2$, 1.7 Hz, 2H), 3.47 (t. $J = 5.7$ Hz, 4H), 4.22 (s, 2H), 4.59 (dt, $J = 8.1$, 3.2 Hz, 1H), 5.76 (dq, $J = 8.1$, 1.7 Hz, 1H), 6.17 (d, $J = 1.7$ Hz, 1H), 7.26 (dd, $J = 7.2$, 2.1 Hz, 2H), 7.28 (tt, $J = 7.2$, 2.1 Hz, 1H), 7.35 (t, $J = 7.2$ Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta =$ 24.0, 24.8, 26.1, 46.1, 57.0, 100.7, 102.6, 127.2(2C), 127.6, 128.7(2C), 129.9; 134.8, 137.9, 171.6.

4a:[a] ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 1.80$–1.92 (m, 4H, overlapped), 3.44–3.53 (m, 4H, overlapped), 3.93 (dd, $J = 3.6$, 1.6 Hz, 2H), 4.18 (s, 2H), 4.94 (dt, $J = 9.9$, 3.6 Hz, 1H), 6.22 (dq, $J = 9.9$, 1.6 Hz, 1H), 7.14 (br s, 1H), 7.25–7.40 (m 5H, overlapped).

4b:[a] ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.41$–1.73 (m, 6H, overlapped), 3.38–3.55 (m, 4H, overlapped), 3.93 (dd, $J = 3.6$, 1.5 Hz, 2H), 4.17 (s, 2H), 4.93 (dt, $J = 9.8$, 3.6 Hz, 1H), 5.98 (dq, $J = 9.8$, 1.5 Hz, 1H), 7.06 (brs, 1H), 7.18–7.40 (m, 5H, overlapped).

4c:[a] ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 3.50$–3.75 (m, 8H, overlapped), 3.95 (dd, $J = 3.6$, 1.5 Hz, 2H), 4.19 (s, 2H), 4.94 (dt, $J = 9.7$, 3.6 Hz, 1H), 5.96 (dq, $J = 9.8$, 1.5 Hz, 1H), 7.12 (br s, 1H), 7.28–7.42 (m, 5H, overlapped).
4d:[b] ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 3.01$ (s, 6H), 3.93 (dd, $J = 3.6$, 1.5 Hz, 2H), 4.17 (s, 2H), 4.93 (dt, $J = 9.9$, 3.6 Hz, 1H), 6.06 (dq, $J = 9.9$, 1.5 Hz, 1H), 7.07 (br s, 1H), 7.20–7.40 (m, 5H, overlapped)

[a] Data from the spectra of mixtures that were obtained by reduction of **2a**–**c** with $NaBH_4$ and contained large amounts of **4a**–**c**. [b] Data from the spectrum of the mixture with **3d**.



Scheme 2. GAPDH- and LDH-type model transfer reactions.

Finally, we performed sequential GAPDH- and LDH-type model reactions in a one-pot reaction (Scheme 2). After removal of the THF solvent, the crude deuterated dihydronicotinamides (**3a**, [D_1]-**3a**, and [D_2]-**3a**) from the GAPDH-type reaction of **2a** with [D_1]-**1** (5 equiv) were used for the following LDH-type reaction with methyl benzoylformate (**5** (5 equiv) in acetonitrile without further addition of Mg^{2+}.[17] After the reaction mixture was stirred for four days at room temperature, the lactate analogue methyl mandelate (**6**) was obtained in 47% yield (based on **2a**) with an H:D ratio of 73:27, as determined by ^{1}H NMR spectroscopy. We then investigated the reduction of **5** with [D_1]-**3a** that was prepared independently from **2a** and sodium dithionite in D_2O in the presence of $Mg(ClO_4)_2$.[18] Partially deuterated **6** was obtained with an H:D ratio of 75:25. This value is in good agreement with that obtained in the model transfer reactions, in view of the ratio of the amounts of **3a**, [D_1]-**3a**, and [D_2]-**3a** formed in the preceding reaction. These findings clearly show that this preferential deuterium/hydrogen transfer from [D_1]-**1** to **6** was mediated by a redox cycle of the NAD^+/NADH model system, as in the real coenzyme system in anaerobic glycolysis.

Consequently, we have accomplished, to the best of our knowledge, the first GAPDH-type oxidation of aldehyde to carboxylate in an NAD^+/NADH model system and have demonstrated its application to model transfer reactions of the GAPDH- and LDH-type. Biomimetic oxidation involving such simple model compounds as **1** and **2a**–**d** is virtually equivalent to the corresponding biological reaction in terms of mechanism and selectivity. The details of the mechanism, the scope and limitation of the reaction, and the application to catalytic model system are under investigation.

Experimental Section

Reaction of **2a**–**d** with **1** (representative procedure): A solution of **1**, prepared from formic acid (18.9 μL, 0.50 mmol) and a solution of EtMgBr in THF (1.0 M, 1.0 mL, 1.00 mmol), was added dropwise by syringe to a suspension of **2a** (34.7 mg, 0.10 mmol) in THF (0.3 mL) at room temperature. The mixture was heated at 90 °C for 1 h under an argon atmosphere. Water was added, and the mixture was extracted three times with diethyl ether. The combined organic phase was dried over $MgSO_4$ and concentrated in vacuo to give a 91:9 mixture of **3a** and **4a** (24.7 mg, 92%). The mixture was further purified by column chromatography on aluminum oxide with deoxygenated hexane/ethyl acetate (1/1); yield: 16.6 mg, 62%.

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[11] Reduction of **2a – d** with $NaBH_4$ in ethanol also afforded a significant amount of 1,6-dihydronicotinamides, the ^1H NMR spectra of which are identical to those of **4a – d**. All the signals (5H) of their dihydronicotinoyl rings were well separated from those of the other products such as **3a – d.**

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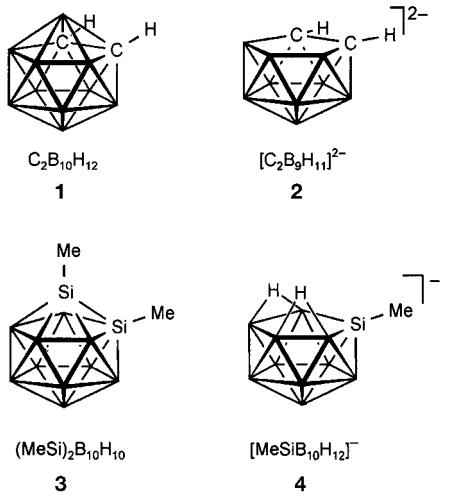
[17] The reaction of **2a** with **1** is expected to generate one equivalent of $MgBr_2$.

[18] The NADH model compound **3a** did not reduce **5** to **6** in the absence of magnesium ions. Therefore, the preceding GAPDH-type model reaction must provide an activator such as $MgBr_2$ for the following LDH-type model reaction.

A Surprising Adduct of a *closo* Cluster**

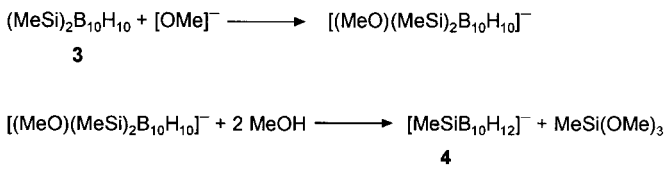
Lars Wesemann,* Yves Ramjoie, Michael Trinkaus, Beate Ganter, and Jens Müller*

In 1968 Hawthorne et al. published the preparation and characterization of 7,8-dicarba-*nido*-undecaborate(1 –) (**2**), the product of the nucleophilic degradation of *o*-carborane (**1**) (Scheme 1).[1] The reaction of dicarba-*closo*-dodecaborane(12) with potassium hydroxide in ethanol gave the eleven-vertex cluster anion **2**. An adduct formed from the attacking ethoxide anion and the neutral *closo* cluster was formulated as a possible reaction intermediate. In *o*-silaborane[2] (**3**), the silicon atoms are unique in that they are incorporated in the skeleton of a borane cluster framework. We are interested in the reactivity patterns of this icosahedral cluster and especially in the silicon centers. The first reaction of *o*-silaborane to be explained was the nucleophilic degradation.[3] Removal of a silicon vertex from the icosahedral cluster results in almost quantitative isolation of the sila-*nido*-undecaborate(1 –) (**4**). This eleven-vertex cluster is a versatile starting material for the synthesis of transition metal complexes with interactions between the silicon and the metal.[4, 5]



Scheme 1. *o*-Carborane and *o*-silaborane and their degradation products.

In this paper we present first insights into the nucleophilic degradation of *o*-silaborane. Attack of one equivalent OH^- in H_2O, MeO^- in MeOH, or pure NH_3, results in the removal of a silicon vertex from the *closo* cluster **3**.[3] According to a plausible mechanism for the degradation, in the first step an adduct is formed comprising the nucleophile and the *closo* cluster (Scheme 2). This adduct should then react with a protic solvent to form the isolated reaction product.



Scheme 2. Nucelophilic degradation of o-silaborane **3** to sila-*nido*-undecaborate(1 –) **4**.

In order to verify the existence of the adduct, we studied nucleophilic attack on **3** in an aprotic solvent by NMR spectroscopy. The cluster **3** was treated with one equivalent of $LiNEt_2$ in THF, and the ^{11}B NMR spectrum (Figure 1) of the reaction mixture shows the quantitative formation of a new compound. Four signals in the ^{11}B NMR spectrum corresponding to ten boron atoms indicate that the product still has C_{2v} symmetry. A shift of the ^{29}Si signal from – 38 to – 76 ppm

[*] Priv.-Doz. Dr. L. Wesemann, Dr. J. Müller, Dipl.-Chem. Y. Ramjoie, Dipl.-Chem. M. Trinkaus, Dr. B. Ganter
Institut für Anorganische Chemie der Technischen Hochschule
Professor-Pirlet-Strasse 1, D-52056 Aachen (Germany)
Fax: (+49) 241-888-8288
E-Mail: lars.wesemann@ac.rwth-aachen.de
jens.mueller@ac.rwth-aachen.de

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