Table 1. Selected analytical data for **2** and **9a–d**.

2: red liquid; elemental analysis calcd(%)for $C_{20}H_{34}N_2O_2$: C 72.28, H 9.63, N 8.43; found: C 72.23, H 9.83, N 8.46. **9a**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.2$ (s, 1H; CH), 5.1 (s, 1H; CH), 1.5 (s, 9H; CH_3); UV/Vis (EtOH): λ_{max}[nm] (ε) = 443 (1800), 246 (16000); high resolution MS (FAB): *m*/*z* (%): 364.2589 (21) [M^++2H], 363.2536 (40) [M^++H], 362.2450 (27) [M^+]. **9b**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 10.4$ (s, 1H; CH), 8.5 (s, 1H; CH), 1.6 (s, 9H; CH_3); UV/Vis (EtOH): λ_{max}[nm] (ε) = 440 (4600), 285 (*shoulder*, 4000), 242 (21000); high resolution MS (FAB): *m*/*z* (%): 363.2528 (100) [M^++H], 334.2135 (82.6) [$M^+ - C_2H_4$]. **9c**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.43$ (s, 1H; CH), 8.5 (s, 1H; CH), 5.9 (s, 1H; CH), 1.8 (s, 9H; CH_3); high resolution MS (FAB): *m*/*z* (%): 364.2594 (63) [M^++2H], 363.2518 (60) [M^++H], 362.2451 (51) [M^+], 348.2657 (73). **9d**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.8$ (s, 1H; CH), 9.1 (s, 1H; CH), 5.6 (s, 1H; CH), 1.8 (s, 9H; CH_3); high resolution MS (FAB): *m*/*z* (%): 362.2804 (12) [M^++CH_4], 348.2655 (22) [M^++2H], 347.2577 (44) [M^++H]

The use of this spin-trap for the in vivo detection of NO is more problematic because of the presence of reducing agents: when **2** was treated with ascorbic acid and the reaction monitored by EPR spectrocopy, the typical spectrum of **9e** (Scheme 2 where X = OH, $Y^1 = Y^2 = H$) immediately appeared and then decayed, but, as expected, at a much lower rate.[8] However, it is possible to distinguish between the NO adducts and **9e** by combining different analytical methods such as mass spectrometry, ^{1}H NMR spectroscopy,[7] (for **9a** and **9b**) and probably by high-field EPR spectroscopy.[15]

Analogously other spin-labeled spin-traps may be used to study the reactions of a radical R_T with reactive radicals.[16]

Received: January 31, 2000
Revised: September 25, 2000 [Z14623]

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Novel NO Biosensor Based on the Surface Derivatization of GaAs by "Hinged" Iron porphyrins**

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Nitric oxide (NO) plays an important role in biology as a mediator, for example, of the endothelial-derived relaxing factor (EDRF)—an agent responsible for the regulation of blood vessel relaxation and for the maintenance of blood pressure.[1] Concentrations of 30 to 300 ppm of NO (1 ppm ≈ 3.3×10^{-5} M) are sufficient to activate the guanylyl cyclase signaling cascade.[2] Since NO is a gaseous and highly reactive species, its direct detection at low concentrations is difficult. The methods used to sense NO have generally been based on following changes in the UV/Vis spectra or electrochemical properties of the sensing elements.[3] Electron paramagnetic resonance (EPR) and NMR image visualization of the distributions of NO free radicals in vivo were also reported, but were found to be limited by spatial resolution and sample size.[4, 5] We report here a novel approach for the direct detection of low concentrations of NO free radicals in physiological aqueous solution (pH = 7.4).

The Molecular Controlled Semiconductor Resistor (MOCSER; see reference [6]) provides an excellent way to

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[**] We thank Rachel Lazar for her help in the synthesis. This work was supported by the Israel Ministry of Sciences Tashtyoth program (D.C., R.N., and A.S.), and by a grant from the Fussfeld foundation (D.C. and A.S.). A.S. holds the Siegfried and Irma Ullman professorial chair.

detect NO. Recently we showed that the electrical resistivity of this device is controlled by the adsorption of molecules on a GaAs semiconductor surface.[6, 7] Here we present a modified version of the MOCSER in which the GaAs surface is derivatized by two-component molecular systems prior to the experiment. Changes in the MOCSER's current when NO binds to the outer molecular component are examined to follow the binding events in situ.

In these systems iron(III) tetraphenylporphyrin chloride (FeTPPCl) moieties, the NO binders, are not bound directly to the GaAs surface, but rather, they are "hinged" remotely from the surface by bifunctional ligands (Scheme 1). These ligands bind axially, through imidazolyl groups, to the fifth and sixth coordination sites of the iron(III) porphyrin, and simultaneously to the GaAs surface of the MOCSER through anchoring groups—disulfides or carboxylic acids. A special feature of such a configuration is that the iron porphyrins will, on the average, be oriented perpendicular to the surface. The axial ligation to the metalloporphyrin rings prevents $\pi-\pi$ interactions between them—interactions that are expected to decrease the porphyrin activity.[8] The imidazole rings of the ligands are bound to the Fe^{III} center in a kinetically labile fashion, so that one of them can be replaced by a stronger binding substrate that approaches near the porphyrin.[9]

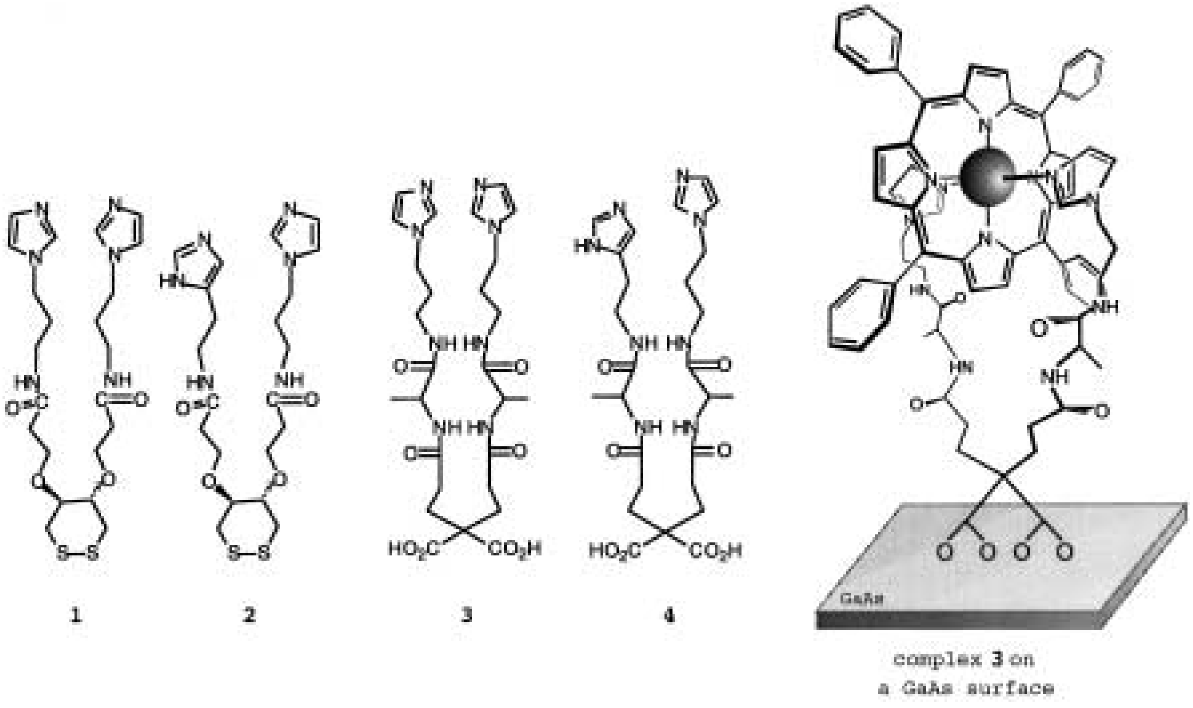
Complexes of FeTPPCl with the four bifunctional ligands shown in Scheme 1 were used to probe the molecular features that may affect the sensing event. Ligand **1** has a symmetric bidentate metalloporphyrin binding site made of *N*-alkylimidazoles and a disulfide as the anchoring group; ligand **2** has the same anchor, but with a nonsymmetric metalloporphyrin binding site, in which the NH group of the imidazole ring imposes a weaker binding of the *N*-alkylimidazole group at the opposite face; ligand **3** is symmetric in binding to the iron porphyrin, but it has a malonic acid as an anchor which binds more strongly to the surface.[7] Modification in both elements relative to **1** were done in ligand **4**, which contains the nonsymmetric binding site for iron porphyrins and a malonic acid anchor. Devices covered by the iron porphyrin complexes of ligands **2**–**4** were expected to be more sensitive to NO than those covered by the corresponding complexes of the "parent" ligand **1**.

The formation of the expected low-spin ligand–FeTPPCl complexes was evidenced in solution by UV/Vis and ^{1}H NMR measurements, as described earlier for ligand **1**.[8] Freshly formed complexes of the four ligands with FeTPPCl were adsorbed onto single crystal GaAs and characterized by FT-IR spectroscopy, using the etched, bare (but oxidized) GaAs as a reference. The main absorbance bands (1700–900 cm^{-1}) are listed in Table 1. For comparison, the corresponding bands of the complexes in KBr are also listed. The typical vibrations of the carbonyl group of the amide moieties and of the ether bonds (in the complexes formed from ligands **1** and **2**) were observed at approximately 1650 and 1100 cm^{-1}, respectively. Strong and broad bands were observed at 1625–1690 cm^{-1} in the spectra of the complexes formed from **3** and **4** because

Table 1. Main assignments of the vibrations in the transmission FT-IR spectra (cm^{-1}) for complexes of **1**–**4** on single-crystal GaAs(100) surfaces. The values obtained in KBr are given in parenthesis.

Complex	$\tilde{\nu}_{CONH}$	$\tilde{\nu}_{COOH}$	$\tilde{\nu}_{C=C}$	$\tilde{\nu}_{aromatic}$	$\tilde{\nu}_{C-O}$
1	1660 (1653)		1551 (1558)	1440 (1442)	1100 (1096)
2	1651 (1652)		1595 (1597)	1441 (1440)	1093 (1094)
3	1630–1690[a] (1653)	(1715)	1546 (1539)	1439 (1441)	1093 (1094)
4	1626–1683[a] (1670)	(1716)	1552 (1554)	1441 (1440)	1137 (1136)

[a] Broad peak overlapping $\tilde{\nu}_{COO^-}$ vibration at ≈1640 cm^{-1}.



Scheme 1.

bands of the carbonyl group of the amide moieties overlapped those of the carboxylate groups. The disappearance of the band corresponding to the carboxylic group in the spectrum ($\tilde{\nu}_{COOH}$ (vibration) 1715–1716 cm^{-1} in KBr) when complexes derived from **3** or **4** were adsorbed onto the GaAs indicates that their carboxylic groups bind to the GaAs surface.[10] The intensities of the absorbance peaks of $\tilde{\nu}_{CONH}$ or $\tilde{\nu}^{as}_{COO^-}$ were about $0.5-1.5 \times 10^{-3}$, which indicates that a film of about one monolayer is formed by each of the complexes (from ligands **1**–**4**) on the GaAs substrate.[11] The ratios of the intensity of the vibrations corresponding to the ligands to those of the iron porphyrins remain similar to the ratios obtained from the KBr measurements, thus eliminating the possibility that the complexes decomposed during the adsorption process.

Contact potential differences (CPD) were measured under photosaturation conditions, to reveal the surface band bending (BB) of the bare GaAs surface, and of surfaces covered with monolayers of the complexes formed with ligands **1**–**4**.[12] Adsorption of the two complexes formed from ligands with disulfide anchors (**1** and **2**) resulted in a small decrease in the BB relative to the bare surfaces. After adsorption of the complexes formed from ligands **3** or **4**, a much more significant decrease in the BB was observed,[13] which was ascribed to the stronger binding of their carboxylic groups, relative to the disulfide groups, to the surface.

The response of MOCSERs covered by the complexes formed from **1**–**4** to different concentrations of NO was determined in aqueous buffer solutions (pH = 7.4) at 25 °C. A constant voltage of 100 mV was applied between the ohmic contacts of the MOCSER during the experiment. Figure 1 shows the changes in the current with time for MOCSERs covered by complexes derived from **1**, when four different concentrations of the organic NO precursor, 1-hydroxy-3-methyl-3-(methylaminopropyl)-2-oxo-1-triazene, were released into the solution. At physiological pH this reagent releases quantitatively two equivalents of NO with first order kinetics and $t_{1/2} = 10.1$ minutes at room temperature.[14–16] As a consequence of the slight differences in the sensing area and light conditions, the current was normalized by dividing the measured current values by the different absolute responses of the complex-covered MOCSERs to the aqueous medium without NO.[17, 18] In less than ten minutes following the introduction of NO no further changes in current were observed, and the saturation current increases with an increase of the NO "steady state" concentration.[19]

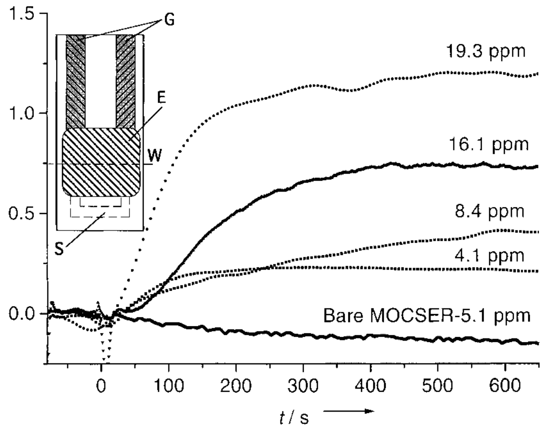


Figure 1. Changes in current over time on exposure of a MOCSER covered by complexes derived from **1** to different concentrations of 1-hydroxy-3-methyl-3-(methylaminopropyl)-2-oxo-1-triazene that releases NO. The lower plot refers to reference measurements with a bare MOCSER. All measurements were done at room temperature, in pH = 7.4 buffer solution, and under N$_2$. Inset: schematic drawing of the sensing surface of the MOCSER. S: sensing window (0.3 mm^2), E: epoxy, G: AuGeNi ohmic contacts, W: water level.

The experimental system was kept under anaerobic conditions (N$_2$) for 30 minutes before and during the experiment to remove practically all the O$_2$ and to prevent formation of the undesired NO$_2$. No changes in current were observed when MOCSERs covered by the complexes were exposed to the unreactive by-product of the proteolysis, that is, *N*,*N*-dimethyl-1,3-propylamine.[14] Only a very slight decrease in current was observed when bare MOCSER devices were exposed to various precursor concentrations (0.5–5 ppm). The changes in current with time for the bare MOCSER with 5 ppm of the precursor are included in Figure 1. The less stable current obtained in this measurement shows that adsorption of the complexes stabilizes the device surface.

Figure 2 shows the linear correlation of the saturation current (normalized) of MOCSERs covered by complexes of ligands **2**–**4** as a function of the NO "steady-state" concentration. To compare the sensitivity of the different MOCSER types toward NO, we followed the (interpolated) current values on exposure of each to 2.0 ppm of NO. This value was below our signal/noise (data not shown) for the MOCSER

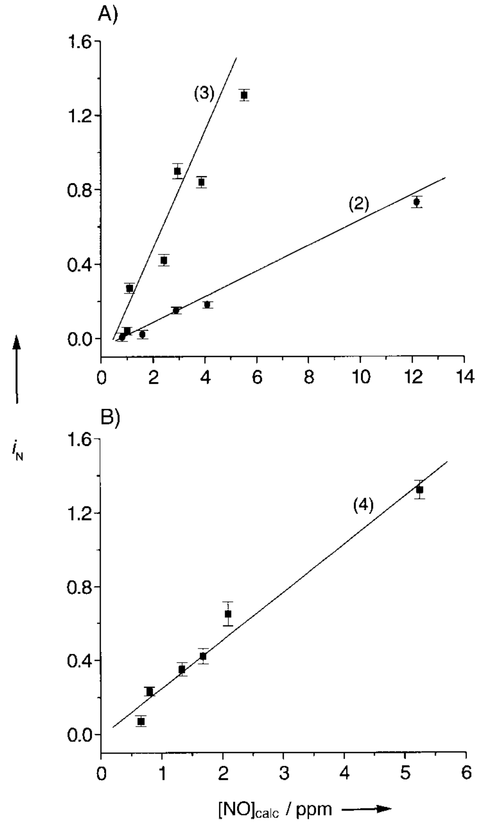


Figure 2. Normalized current of MOCSERs covered by complexes derived from **2** and **3** (A), or **4** (B), as a function of the NO "steady-state" concentration.[19] Different MOCSERs were used for each measurement.

covered by the complex with **1**, and the normalized current values[17] for MOCSERs with complexes formed from **2**, **3**, and **4** were 0.07, 0.38, and 0.50, respectively.

Two methods were used to release NO from the saturated sample (complex with **3**, 5.0 – 10.0 ppm NO) and to regenerate the ability of the MOCSER to detect NO. In the first one, the NO-saturated sample was taken out of the buffer solution, dried in a stream of dry N_2 for less than 15 seconds, and reimmersed in a clean buffer solution. A current typical for the NO-free system could then be measured. In the second method, the NO-containing solution was replaced by a clean buffer solution, and the current of the NO-free system was reached within a few minutes ($t_{90} \approx 120$ s). Subsequent exposure of the samples to the same concentration of NO resulted in the (saturation) current being 70 – 85 % of the original change, which demonstrates the system is reasonably reversible under these conditions.

The system responds to NO mainly through the binding of NO to the iron(III) porphyrin moieties,[20] and not by direct binding to the surface. The increase in current when the NO binds to the derivatized surfaces results from a decreased potential drop across the conducting GaAs layer (of the device). Such a decrease is explained by a change in the dipole of the iron porphyrin/ligand complex following the replacement of one coordinated imidazole ring by the NO radical (stronger positive monopole at the binding group).[21]

Higher sensitivity was obtained for MOCSERs with the nonsymmetric complex formed from **2** than with the complex of **1**, as deduced from the detection values of 2.0 ppm of NO. This result can be understood from the greater ease by which NO replaces the *N*-alkylimidazolyl ring of ligand **2** relative to the imidazolyl ring of ligand **1** as a chelator to the Fe^{III} center. A similar process was observed earlier for replacement of the coordinated *N*-alkylimidazole ring by O_2.[22] Devices covered by the complexes formed with ligand **3** are also more sensitive to NO than those covered by complexes derived from **1**. Since the Fe^{III} binding sites in the two complexes are the same, this enhancement of the signal with **3** is attributed mainly to a stronger binding of the dicarboxylate group to the surface, relative to the binding of the disulfide group.[23] The tighter mode of binding is expected to improve the electrical communication between the anchoring site on the GaAs surface and the NO binding site. Such communication can be considered as signaling through the ligand skeleton, via its anchor, to the surface.

Devices covered by the complexes of **4** were found to be the most sensitive to NO. By utilizing the complex formed with **4** we demonstrated simultaneously: a) a better NO binding to FeTPPCl, relative to the complex with **3**, occurs with the nonsymmetric metalloporphyrin binding site, and b) an enhanced signaling to the GaAs surface via the dicarboxylic anchors occurs, relative to that found in devices covered by the complexes formed with **2**. We note that the currents obtained when MOCSERs covered by complexes derived from **4** were exposed to NO are similar to the summation in current obtained in the two separate measurements with devices covered by the complexes derived from **2** or **3** (for example, for 2.0 ppm of NO). This phenomenon suggests that the symmetry of the metalloporphyrin binding site and the type of anchoring group dictate independently the activity of the entire system.

In recent, preliminary, experiments we observed that dry devices covered by complexes from **1** or **2** are much more sensitive to NO(g) than to O_2(g) or CO(g). This result is reasonable if one considers the weaker binding of O_2(g) and CO(g) to iron(III) porphyrins.[24] Experiments, designed to sense NO exclusively in a biological environment of competing ligands, for example, other NO_x species, are under way.

Experimental Section

Synthesis: The synthesis of ligands **1** and **2** was described earlier,[8, 22] and the detailed procedure to make ligands **3** and **4** will be described soon.[25] Characterization of **3:** IR (KBr): $\tilde{\nu} = 1652$ (CONH), 1715 cm^{-1} (COOH); ^1H NMR (CD_3OD): $\delta = 8.20$ (s, 2H), 7.30 (s, 2H), 7.10 (s, 2H), 4.12 (br, 6H), 3.19 (t, $J = 7.0$ Hz, 4H), 2.19 (t, $J = 6.5$ Hz, 4H), 2.12 (t, $J = 6.5$ Hz, 4H), 1.99 (quint, $J = 7.0$ Hz, 4H), 1.30 (d, $J = 8.0$ Hz, 6H); FAB-MS: m/z: 604.8 $[M+H]^+$. **4:** IR (KBr): $\tilde{\nu} = 1670$ (CONH), 1716 cm^{-1} (COOH); ^1H NMR (CD_3OD): $\delta = 7.76$ (s, 2H), 7.18 (s, 1H), 6.99 (s, 1H), 6.90 (s, 1H), 4.24 (dq, 2H), 4.06 (t, $J = 6.5$ Hz, 2H), 3.35 (t, $J = 7.0$ Hz, 2H), 3.16 (t, $J = 7.0$ Hz, 2H), 2.79 (t, $J = 7.0$ Hz, 2H), 2.21 (br, 4H), 2.14 (br, 4H), 1.98 (quint, $J = 7.0$ Hz, 2H), 1.26 – 1.34 (dd, 6H); MS (electrospray): m/z: 591.4 $[M+H]^+$.

Complexes of each ligand with FeTPPCl were made by mixing 1/1 solutions (> 15 mM) in DMF (**1** or **2**), $CHCl_3/CH_3OH$ 9/1 (**3**), or $CHCl_3$/2,2,2-trifluoroethanol (TFE) 8/2 (**4**).

Sample preparation: Etched MOCSERs or GaAs substrates (NH_3/H_2O 1/9) were immersed in solutions of freshly prepared ligand – FeTPPCl complexes overnight. The substrates were then rinsed by $CHCl_3$/hexane (5 % v/v, **1** or **2**) or by $CHCl_3$ (**3** or **4**), and dried (N_2). To prevent leakage of the electrical current through the conducting solution, the device was encapsulated using epoxy, leaving a "sensing window" uncovered (inset to Figure 1). The linearity of the *I-V* characteristics was checked during the measurements by a Keithley 236 Source Measure Unit.

Sensing NO by the derivatized MOCSERs: The NO-release solution was prepared by dissolving a weighted amount of commercial 1-hydroxy-3-methyl-3-(methylaminopropyl)-2-oxo-1-triazene (Sigma) in a 0.01M solution of NaOH. These conditions for preparing the NO solution and for NO injection are very similar to those previously described.[14] At basic pH values the reagent is stable, and at physiological pH it releases 2.0 equivalents of NO with first order kinetics having $t_{1/2} = 10.1$ min at 22 °C. As shown in ref. [14] NO is the only nitrosyl compound obtained during proteolysis.[14] After the MOCSER current had been stabilized in the (pH = 7.4) phosphate buffer solution, the precursor was injected, and the saturation current was obtained within 10 min.

Received: May 17, 2000
Revised: September 5, 2000 [Z15136]

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Stereoselective Synthesis of Highly Functionalized *C*-Glycosides based on Acetal [1,2] and [1,4] Wittig Rearrangements**

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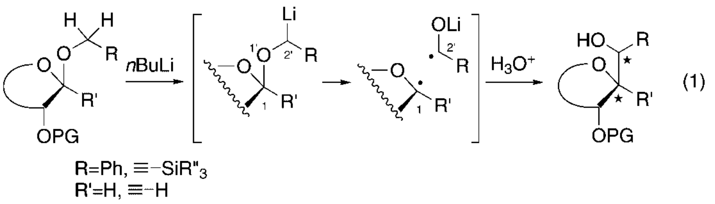
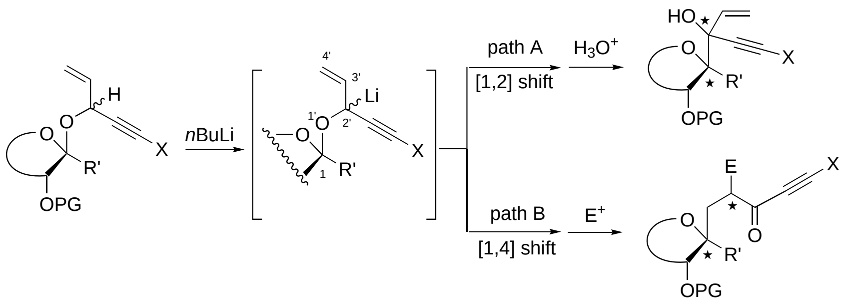
The [1,2] Wittig rearrangement is a classic class of carbanion rearrangements which is now well recognized to proceed by a radical dissociation–recombination mechanism.[1] Despite potentially useful carbon–carbon bond forming reactions, however, synthetic application of this rearrangement still remains severely limited, mainly because of the rather low yields and the restricted range of substrates. To this end, we have recently developed the acetal [1,2] Wittig rearrangement protocol which provides *C*-glycosides from primary-alcohol-derived *O*-glycosides in a highly diastereoselective manner [Eq. (1)].[2, 3]



(1)

To extend this protocol we envisioned that the rearrangement of a more functionalized *O*-glycoside system with a secondary-alcohol-derived migrating terminus might afford the *C*-glycoside with a tertiary alcohol side chain.[4] A key feature for the success of the rearrangement is a rational design of a migrating terminus. Thus, we selected the ethynylvinylmethanol system as the migrating terminus with a view that the ethynyl and vinyl substituent not only enhances the [1,2] Wittig reactivity, as a result of the large radical stabilizing effect, but also imparts unique multifunctionality to the products (Scheme 1, path A). Here we report a stereoselective entry to the highly functionalized *C*-glycosides based on an acetal [1,2] Wittig rearrangement and also a novel [1,4] Wittig rearrangement of an acetal system (Scheme 1, path B), which was discovered by chance in the [1,2] rearrangement study.



Scheme 1. [1,2] and [1,4] Wittig rearrangement approach to the multi functionalized *C*-glycosides.

At the outset we studied the rearrangement of acetal *β*-**3** which was prepared from (−)-dihydro-3-hydroxy-4,4-dimethyl-2(3*H*)-furanone ((−)-pantolactone) as a 1:1 epimeric mixture at the C1′ position in three steps: protection of the hydroxyl groups, addition of lithium acetylide, and a montmorillonite K 10 clay catalyzed acetalization[5] with the protected ethynylvinylmethanol **2** (racemate) [Eq. (2); TBDPS = *tert*-butyldiphenylsilyl]. Two epimers of *β*-**3** were separable by chromatography on silica gel and their stereochemistries were determined by X-ray crystallography and by chemical conversion.[6] The reaction of *β*-(*R*)-**3** with *n*BuLi (3 equiv) in THF at −78 °C afforded (< 10 min) the [1,2] re-

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[**] This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture, Japan, and by the Research for the Future Program, administered by the Japan Society for the Promotion of Science.

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