of **1a**–**c** were obtained in each case, although only the structure of **1a** is reported here. Satisfactory elemental analyses were obtained for **1a**, **1b**, and **2**. **1a**: Yield >90%; $^{31}P\{^1H\}$ NMR (−50 °C, [D$_8$]toluene) −21.5(s). **1b**: Yield 80%; $^{31}P\{^1H\}$ NMR (−50 °C, [D$_8$]toluene) −27.0(s). **1c**: Yield >90%; $^{31}P\{^1H\}$ NMR (−50 °C, [D$_8$]toluene) −29.9(br.s). Synthesis of **2**: Mercuric acetate (0.63 g, 1.98 mmol) was dissolved with nPr$_3$P (0.64 mL, 4 mmol) in THF (15 mL) and cooled to −65 °C. This clear solution was added to a freshly prepared solution of **1a** with excess nPr$_3$P. Upon warming to −30 °C, the solution became bright orange and, within several hours at this temperature, orange crystals (0.62 g, 60%) of **2** appeared.

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[11] X-ray structure analyses: Enraf-Nonius Kappa CCD diffractometer ($Mo_{K\alpha}$ radiation). Crystal data for $C_{30}H_{72}P_3CuSSi$ **1a**: colorless polyhedron, $M_r = 649.48$, triclinic, space group $P\bar{1}$, $a = 10.9010(2)$, $b = 11.1233(2)$, $c = 17.3734(2)$ Å, $\alpha = 92.8890(11)$, $\beta = 97.3160(11)$, $\gamma = 105.1140(7)^\circ$, $V = 2009.48(6)$ Å^3, at 200 K, $Z = 2$, $\rho_{calcd} = 1.073$ g cm^{-3}, $\mu = 0.761$ mm^{-1}, $2\theta_{max} = 41.6^\circ$, 11440 reflections collected, 4058 independent ($R_{int} = 0.037$). The structure was solved by direct methods and refined on F^2 using SHELXTL software. Data were corrected for Lorentz and polarization effects. No absorption corrections were performed. All atoms (with the exception of disordered carbon sites, see supplementary material) were refined anisotropically and hydrogen atoms were included as riding on their respective carbon atoms, 307 parameters). Final $R = 0.0357$ ($wR_2 = 0.0851$) and GoF = 1.058. Largest difference peak and hole = 0.34 and −0.25 e Å^{-3}, respectively. For $C_{162}H_{378}P_{18}Cu_{20}S_{25}Hg_{15}$ **2**: orange polyhedron, $M_r = 7965.25$, rhombohedral, space group $R\bar{3}$, $a = 23.5814(9)$, $c = 43.376(2)$ Å, $V = 20889.1(15)$ Å^3, at 200 K, $Z = 3$, $\rho_{calcd} = 1.900$ g cm^{-3}, $\mu = 10.044$ mm^{-1}, $2\theta_{max} = 40.0^\circ$, 8646 reflections collected, 4339 independent ($R_{int} = 0.0744$). The structure was solved and refined as for **1a** except C atoms were refined isotropically, P–C and C–C distances were each refined with common values, and H atoms were not included. Final $R = 0.0709$ ($wR_2 = 0.2167$) and GOF = 1.102. Largest difference peak and hole = 1.11 and −1.21 e Å^{-3}, respectively. Atoms Cu3 and Hg3 were statistically distributed over the six equivalent positions ($\bar{3}$) around S5. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-135160 and -135161. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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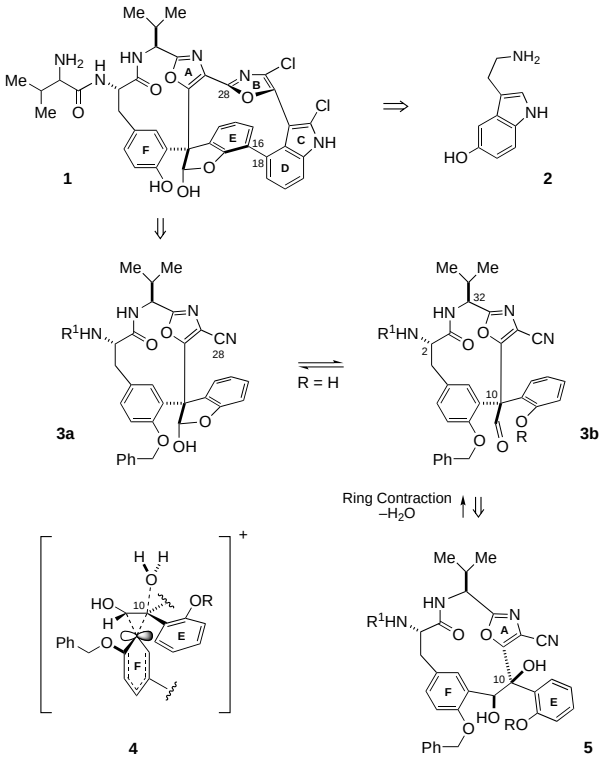
Stereocontrol in Pinacol Ring-Contraction of Cyclopeptidyl Glycols: The Diazonamide C_{10} Problem**

Xin Chen, Lothar Esser, and Patrick G. Harran*

Diazonamide A (**1**) is a unique product of marine invertebrate secondary metabolism reported to inhibit the growth of a human colorectal carcinoma ($IC_{50} < 15$ ng mL^{-1} against HCT-116) in vitro.[1] Nearly a decade after this discovery, the biochemical events mediating this activity, as well as the effects of the molecule on other cell types and tissues, remain unknown. Mode of action studies have been limited by a shortage of natural material[2] and the difficulties inherent in efforts to reconstitute the structure through synthesis.[3] The diazonamide network of linked aromatic/heteroaromatic rings maintains elements of axial chirality (constrained to a single atropisomeric form) around a densely functionalized triarylacetaldehyde core **3b** (Scheme 1). Recently, we



Scheme 1. Diazonamide synthesis: primary disconnections and strategy for control of stereochemistry at C_{10}.

[*] Prof. P. G. Harran, X. Chen, L. Esser[+]
Department of Biochemistry
University of Texas Southwestern Medical Center at Dallas
Dallas, TX 75390-9038 (USA)
Fax: (+1) 214-648-6455
E-mail: pharra@biochem.swmed.edu

[+] Author to whom correspondence regarding X-ray analyses should be addressed.

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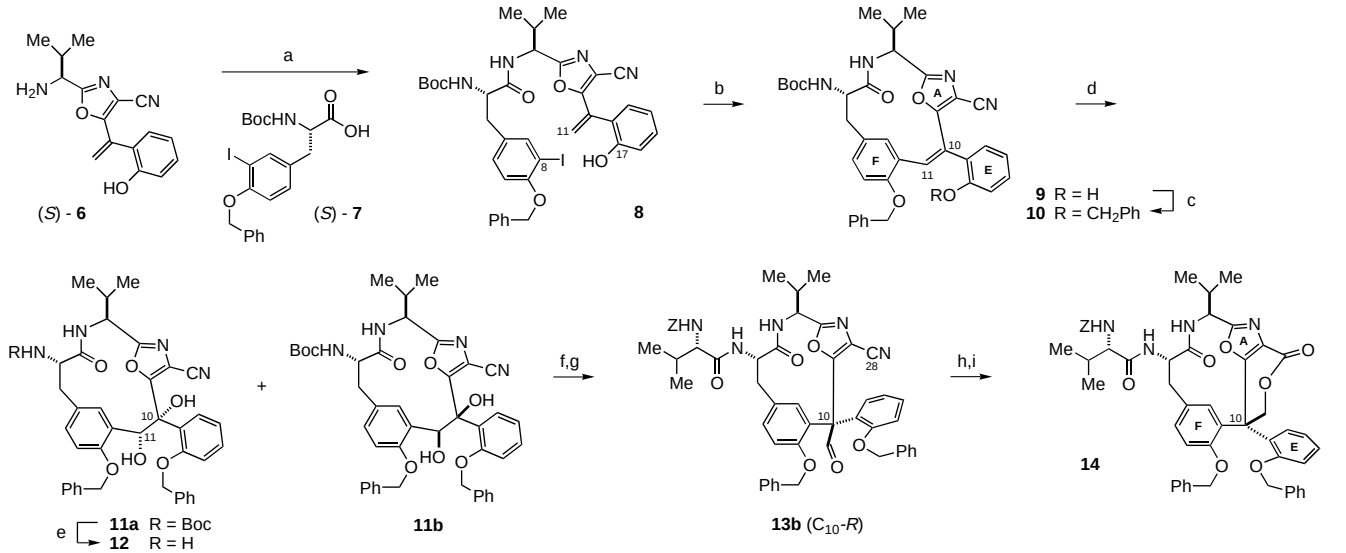
proposed that atropisomer selection in this case could be made dependent on the configuration of a preassembled core; **3a** and/or **3b**.[4] Stepwise annulation of a B/C/D segment onto compounds **3**, initiated with acylation of serotonin (**2**) and culminating in selective formation of the $C_{16}-C_{18}$ biaryl linkage, would generate a skeleton in which the relative rotational orientation of rings A–E paralleled that observed in **1**.

To begin along this pathway, lactam **9** (Scheme 2) was prepared[4] with the intent that the triarylethylene component serve as a latent equivalent of acetaldehyde **3b**. Glycol **5**, an oxidized congener of **9**, was targeted as a direct precursor to **3b** in that acidolysis of **5** could generate aldehyde **3b** (Scheme 1) by a ring-contracting pinacol rearrangement. Provided kinetic control over the process was established, rearrangement through the geometry of a bridging phenonium ion[5] **4** (Scheme 1) would communicate C_{10} stereochemistry (with inversion) from **5** to **3b** independent of the relative configurations at C_2 and C_{32}. We outline here an implementation of this strategy as well as additional maneuvers in preparation for the final stages of a diazonamide total synthesis.

Lactam **9** is derived from two primary fragments with a catalyzed cyclization forming the C_8-C_{11} bond (**8**→**9**, Scheme 2). The sequence remains as described in the literature[4] except in the execution of the Heck endocyclization. The original procedure used 10 mol% of $[Pd_2(dba)_3]$ and 2 equivalents of Ag_3PO_4 (refluxed in THF for 11 h) convert **8** into **9** in 66% yield. This method was, however, limited as the reaction would often stall (with deposition of metallic palladium) and would invariably generate by-products at high conversion. Incorporation of 2-(di-*tert*-butylphosphanyl)biphenyl into the mixture (1:1 Pd atom/phosphane)[6] generates a more robust catalyst (ostensibly not more active) that permits the same transformation to be executed in higher yield with less metal. Notably, all other phosphane additives which were examined inhibit the reaction.

With **9** in hand, the E-ring phenol was etherified and attention turned towards dihydroxylating the $C_{10}-C_{11}$ olefin. Compound **10** resisted attempts at catalyzed oxidation, both for dihydroxylation and epoxidation. Fortunately, stoichiometric osmylation[7] was effective and afforded stable osmium glycolates which could be purified by chromatography. Low-temperature treatment with H_2S[8] subsequently liberated isomeric diols **11a** and **11b** (60% combined yield) although assignment of the relative stereochemistries of **11a**, **b** proved difficult. Efforts to predict the facial bias of lactam **10** towards achiral reagents by calculation of conformational preference, or to directly assign configuration with spectroscopic data, were inconclusive. In fact, the solid-state structure of **9**[9] readily superimposed onto the lowest energy conformers calculated for its benzylated derivative **10** (Figure 1a) and ^{1}H NMR did not detect, in either **9** or **10**, conformational equilibria within the range $-20\,^\circ$C to $60\,^\circ$C. However, while the slightly cup-shaped topography of lactam **10** appeared to favor oxidation from the β-face (as drawn); its "*exo*"-orientated F-ring benzyloxy substituent seemed to shield along this same trajectory.

A crystal of **11a** suitable for X-ray diffraction finally allowed an unambiguous assignment.[9] The result (Figure 1b) was instructive in several ways. Compound **11a** was the major diol isomer ($ds = 5{:}1$) and its C_{10} hydroxyl group was α-oriented. If the original proposition that diols of this type would rearrange with inversion at the migrating terminus was correct, α hydroxyl group stereochemistry at C_{10} in **11a** would translate into unnatural *S* stereochemistry at C_{10} in pinacol products (such as C_{10} *epi*-**3b**). Moreover, the C_8-C_{11}-C_{10}-O_9 torsional angle in **11a** is 172.2° and the plane of the F-ring is roughly perpendicular to the ring containing atoms C_{11}, C_{10}, and O_9—a favorable arrangement for ring-contraction via



Scheme 2. a) TBTU, DIPEA, DMF, RT (89%); b) $[Pd_2(dba)_3]$ (3 mol%), 2-(di-*tert*-butylphosphanyl)biphenyl (6 mol%), Ag_3PO_4, THF (0.025 M), 90 °C, 69%, 82% based on recovered **8**; c) DEAD, PPh_3, $PhCH_2OH$, THF, 97%; d) 1. OsO_4, DMAP (2 equiv), *t*BuOH/H_2O, RT; 2. H_2S, THF, $-50\,^\circ$C, 57–60%, **11a**:**11b** = 5:1; e) *p*TsOH (1 equiv), toluene, 95 °C, 1 h, 90%; f) *p*TsOH (3.0 equiv), toluene, 95 °C, 2.2 h; g) *N*-Z-L-Val-OH, TBTU, DIPEA, DMF, 25% from **11b**; h) $NaBH_4$, MeOH/THF, $-10\,^\circ$C, 82%; i) (+)-10-camphorsulfonic acid (1 equiv), PhH, 45 °C, 24 h, 57%. TBTU = *O*-Benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate, DIPEA = *N*,*N*-diisopropylethylamine, RT = room temperature, dba = dibenzylideneacetone, DEAD = diethylazodicarboxylate, DMAP = 4-dimethylaminopyridine, Z = benzyloxycarbonyl, Val = valine, Boc = *tert*-butoxycarbonyl.

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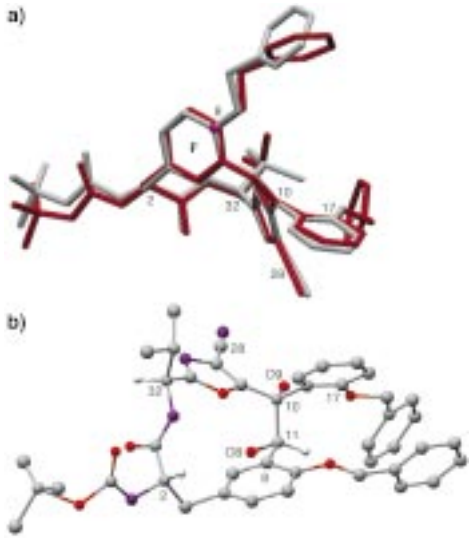
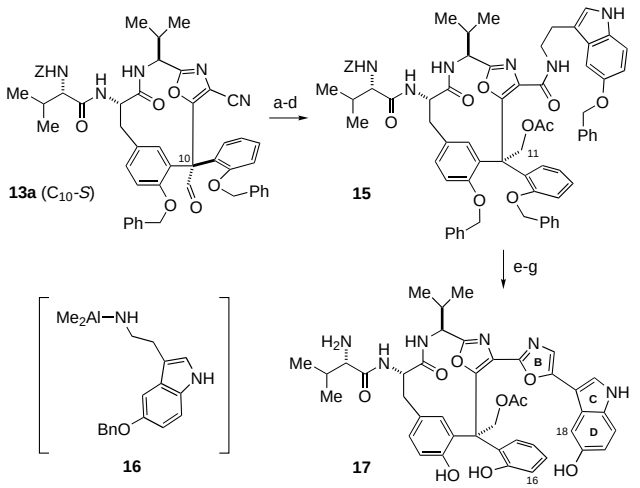


Figure 1. a) Rigid superimposition (RMSD root mean square deviation = 0.131 Å) of lactam ring atoms in the X-ray structure of **9**[9] (blue) and the lowest energy conformer calculated for its benzylated derivative **10** (red). The diagram is oriented to highlight topography in the macrocycle. b) X-ray structure of **11a**.[9] Hydrogen atoms are partially omitted for clarity. Calculations performed and images generated with Macromodel V6.0.



Scheme 3. a) $NaBH_4$, MeOH/THF, −10 °C, 85 %; b) (+)-10-camphorsulfonic acid (1.4 equiv), PhH, 60 °C, 6.5 h, 81 %; c) **16**, toluene/CH_2Cl_2, RT, 88 %; d) Ac_2O, pyridine, CH_2Cl_2, 0 °C, 95 %; e) DDQ, THF/H_2O, RT, 92 %; f) PPh_3, $(CCl_3)_2$, Et_3N, CH_2Cl_2, RT, 94 %; g) Pd/C (10 %), HCO_2H, Et_3N, MeOH/H_2O, RT, 90 %. DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

charge-delocalized species ent-**4** (Scheme 1) upon O_9 protonation.

Experiments to test this idea encountered an unexpected hydrolytic stability of both **11a** and **11b**. The C_2 *tert*-butyl carbamate of either molecule was cleanly decomposed (>90 % yield of corresponding amino diols) using trifluoroacetic acid, HCl or, preferably, *p*TsOH (*p*-toluenesulfonic acid). More forcing conditions were required to initiate pinacol chemistry. For example, the product obtained from reacylation of the C_2 amine of **12** with *N*-Z-L-Val-OH (TBTU, DIPEA, DMF, 85 % yield from **11a**) underwent rearrangement in the presence of 2.5 equivalents of anhydrous *p*TsOH at 95 °C. A 35 % yield of aldehyde **13a** was afforded (Scheme 3) along with one major by-product (≈35 %) which data indicates is a ketone resulting from either C_{11} hydride migration or semi-pinacolic rearrangement. Importantly, a single aldehyde isomer was observed in crude ^{1}H NMR spectra and the X-ray structure of crystalline **13a** showed that the stereochemistry at C_{10}, derived from **11a**, was indeed *S*.[9] Stereochemical fidelity was also observed in the rearrangement of **11b**. In this case, Boc removal and pinacol rearrangement were carried out in one flask (3.0 equivalents *p*TsOH, toluene, 95 °C) and the resultant crude residue acylated with *N*-Z-L-Val-OH (TBTU, DIPEA, DMF) to furnish aldehyde **13b** in 25 % overall yield (Scheme 2). One major, as yet unidentified, by-product also accompanied **13b** although there was no evidence for contamination with **13a**. When subjected to strong acid (1.5 equivalents *p*TsOH, toluene, 95 °C) **13a** and **13b** did not interconvert or produce more than a trace of putative ketone by-products.

These results corroborated the working model for stereochemical control in the glycol rearrangement and represent the first synthesis of an intact diazonamide triarylacetaldehyde. Recently, the conversion of **13b** into a form amenable for completion of the diazonamide polycycle has been achieved. A primary concern was how to unmask and activate the C_{28} carboxylate. This issue had been examined in earlier intermediates with no indication that hydrolysis of the nitrile would be viable. A reduction/oxidation sequence was likewise problematic. The solution came with an assisted hydrolysis using, as an internal nucleophile, the alcohol generated from **13b** by $NaBH_4$ reduction. Warming this material with (+)-10-camphorsulfonic acid (C_6H_6, 45 °C, 24 h) produced lactone **14**—presumably via imidate hydrolysis during workup. The lability of this lactone ring allows incorporation of serotonin derivatives, by neutral amidation, and quick entry into late stages of diazonamide total synthesis. In the more abundant C_{10} epimeric series, elaborations have been explored in some detail. For example, the corresponding lactone derived from **13a** combines with dimethylaluminum amide **16**[10] to afford, after acylation, δ-acetoxy amide **15** (Scheme 3). Four-electron oxidation at the indole benzylic position, dehydration of the incipient β-keto amide[3e], and exhaustive hydrogenolysis subsequently generates **17**—a structure appropriately functionalized to examine C_{16}–C_{18} biaryl synthesis by oxidative phenolic coupling.

Experimental Section

13b: Anhydrous *p*TsOH (14 mg, 0.081 mmol) was added to a solution of **11b** (51.1 mg, 0.067 mmol) in dry toluene (4.5 mL) under N_2. The resultant suspension is heated to 95 °C. Two additional portions of *p*TsOH (13 mg each) are added after 60 and 90 min, respectively, while stirring is continued at 95 °C. The mixture is cooled to room temperature, diluted with EtOAc (10 mL), washed with sat. $NaHCO_3$ (2 × 5 mL), and dried over Na_2SO_4. Following concentration in vacuo, the crude residue (49.9 mg) is dissolved in dry DMF (0.6 mL) and cooled to 0 °C. *N*-Z-L-Val-OH (17 mg, 0.068 mmol), iPr_2NEt (12 μL, 0.069 mmol) and TBTU (22 mg, 0.069 mmol) are added successively. The incipient solution is brought to RT over 40 min, diluted with EtOAc (5 mL), washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. Purification by flash chromatography (EtOAc/benzene 3/7) affords **13b** (14.6 mg, 25 %) as a white film; $R_f = 0.27$ (EtOAc/benzene, 3:7); $[\alpha]_D^{25} = -36.0°$ ($c = 1.0$, $CHCl_3$); IR (film): $\tilde{\nu} = 3290$,

2962, 2246, 1708, 1647, 1537, 1500, 1249, 1024, 753 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 60 °C): $\delta = 9.86$ (s, 1H), 7.45 (td, $J = 7.6$, 1.6 Hz, 1H), 7.33 (d, $J = 4.4$ Hz, 4H), 7.29 (m, 1H), 7.22 (m, 6H), 7.15 (m, 4H), 7.04 (m, 2H), 6.97 (t, $J = 7.4$ Hz, 1H), 6.87 (d, $J = 8.4$ Hz, 2H), 6.44 (d, $J = 8.0$ Hz, 1H), 6.11 (d, $J = 7.2$ Hz, 1H), 5.80 (s, 1H), 5.25 (d, $J = 8.8$ Hz, 1H), 5.21 (d, $J = 12.0$, 1H), 5.14 (d, $J = 12.0$ Hz, 1H), 5.08 (s, 2H), 4.93 (s, 2H), 4.64 (t, $J = 7.6$ Hz, 1H), 4.25 (td, $J = 10.0$, 2.4 Hz, 1H), 3.98 (t, $J = 8.0$ Hz, 1H), 3.13 (t, $J = 12.4$ Hz, 1H), 2.61 (d, $J = 11.2$ Hz, 1H), 2.08 (m, 2H), 1.04 (d, $J = 6.8$ Hz, 3H), 0.94 (m, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 195.8$, 172.1, 171.2, 164.6, 158.7, 157.2, 156.5, 154.3, 136.4, 136.0, 135.9, 132.5, 131.5, 130.8, 130.4, 129.3, 128.7, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9, 127.4, 127.2, 122.5, 121.7, 113.6, 113.4, 112.9, 111.7, 71.0, 67.3, 62.4, 60.3, 56.3, 55.3, 53.7, 51.0, 37.9, 31.7, 30.0, 19.6, 19.3, 18.1; MS (Positive electrospray) for $C_{52}H_{52}N_5O_8$ $[M+H]^+$: calcd: 874.4, found: 874.4; HRMS (FAB) for $C_{52}H_{52}N_5O_8$ $[M+H]^+$: calcd: 874.3815, found: 874.3797.

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Electrochemical Transduction of Liposome-Amplified DNA Sensing**

Fernando Patolsky, Amir Lichtenstein, and Itamar Willner*

Dedicated to Professor Heinz Dürr on the occasion of his 65th birthday

The development of DNA sensors has recently attracted substantial attention in connection with research efforts directed at gene analysis, the detection of genetic disorders, tissue matching, and forensic applications.[1, 2] Optical detection of DNA was accomplished by the application of fluorescence-labeled oligonucleotides,[3] or by the use of surface plasmon resonance (SPR) spectroscopy.[4] Electronic transduction of oligonucleotide – DNA recognition events, and specifically the quantitative assay of DNA, are major challenges in DNA-based bioelectronics.[5] Electrochemical DNA sensors based on the amperometric transduction of the formation of double-stranded oligonucleotide – DNA assemblies have been reported.[6] Also, electrostatic attraction or intercalation of transition metal complexes[7] or dyes[8] was used for the voltammetric probing of the production of double-stranded oligonucleotide assemblies. Microgravimetric quartz crystal microbalance (QCM) analyses were also applied to sense the formation of double-stranded oligonucleotide – DNA complexes on surfaces.[9]

Two fundamental issues that need to be addressed for the development of DNA sensors relate to the specificity and sensitivity of the sensing devices. We have reported on a general method to amplify biorecognition and sensing events by the biocatalyzed precipitation of an insoluble product on the electrode support.[10–12] Enzyme electrodes,[10] immunosensors,[11] and specifically DNA sensors[12] were developed by this amplification route. We have also described specific DNA sensing by the application of a three-component double-stranded sensing assembly consisting of a primer oligonucleotide that is complementary to the mutation domain, the analyte DNA, and a labeled oligonucleotide for assaying and amplifying of the recognition event.[12] Here we report on a novel method for the amplification of oligonucleotide – DNA biorecognition events using functionalized liposomes.[13] The DNA sensing events are transduced electrochemically, using Faradaic impedance spectroscopy. Electronic DNA sensors of unprecedented specificity and sensitivity have been organized, and they exhibit a 10^5- to 10^6-fold enhancement of sensitivity over previous electrochemical DNA sensors.[6, 14]

One DNA sensing configuration is outlined in Scheme 1 A. The sulfanylhexyl oligonucleotide **1** was assembled as a monolayer on an Au electrode. The surface coverage (1.1×10^{-11} mol cm^{-2}) was determined by the electrochemical meth-

[*] Prof. I. Willner, F. Patolsky, A. Lichtenstein
Institute of Chemistry
The Hebrew University of Jerusalem
Jerusalem 91904 (Israel)
Fax: (+972) 2-6527715
E-mail: willnea@vms.huji.ac.il

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