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9*H*-Thioxanthen-9-one *S*,*S*-dioxide based redox active labels for electrochemical detection of DNA duplexes immobilized on Au electrodes

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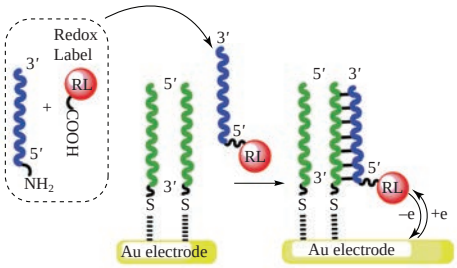
The reaction of 2-bromomethyl-9*H*-thioxanthen-9-one with 2-/4-mercaptobenzoic acids led to 2-/4-[(9-oxo-9*H*-thioxanthen-2-yl)methylthio]benzoic acids. Their further oxidation by H_2O_2 gave the corresponding *S*,*S*-dioxides, which were used as redox active modifiers for a model oligonucleotide. Hybridization of the modified oligonucleotide with a complementary oligoprobe immobilized on Au electrode led to formation of the corresponding redox active DNA duplex, which was reliably detected by cyclic voltammetry.



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Elaboration of devices for electrochemical detection of target DNA hybridization, which are called genosensors, has been in active progress in the last decades.[1–8] Electrochemical genosensors are generally divided into two types, namely reagentless and reagent-based ones.[8] The reagentless sensors typically employ modified electrodes to detect the hybridization,[9–13] while the reagent-based ones deal with redox active enzymes and other electroactive compounds with affinity to ssDNA or dsDNA and demonstrate rapid response as well as operational convenience.[14]

Redox active DNA labels or intercalators in general should have low redox potentials, reversibility of electron transfer in aqueous buffers and must not undergo a non-electrochemical side reaction upon the electron transfer, to avoid their decay during the DNA detection. The main types of compounds, which have been examined as redox active labels in genosensors, are based on ferrocene,[15–18] quinone derivatives,[19,20] transition metal complexes with organic electroactive moieties[21–23] and methylene blue.[24–27] The elaboration of redox active labels with various electrochemical responses, which produce signals at different potential values, constitutes an important task, since it makes possible a parallel multitarget analysis of several DNA sequences.[28]

We have already described the redox active oligonucleotide modifiers based on benzoquinone and naphthoquinone with well-distinguished reduction potentials in the range between the ones for anthraquinone- and ferrocene-based labels.[29] In this work, we report the synthesis and electrochemical investigation of new redox active oligonucleotide modifiers based on the 2-methyl-9*H*-thioxanthen-9-one *S*,*S*-dioxide moiety, which represents a structure related to thioflavone-*S*,*S*-dioxide.[30] The electrochemical reduction (ECR) of 2-methyl-9*H*-thioxanthen-9-one *S*,*S*-dioxide itself is characterized by consecutive transfer of two electrons at different potentials, namely EE-process, as well as by strict reversibility in an aprotic solvent medium for both reduction peaks.[31] The same is true for the ECR of anthraquinone, which makes the *S*,*S*-dioxide a promising precursor for redox active oligonucleotide modifiers.

Oligonucleotide modifiers **2a** and **2b** were synthesized by the K_2CO_3-mediated nucleophilic substitution[32] of bromine atom in 2-bromomethyl-9*H*-thioxanthene-9-one[33] with 2- and 4-mercaptobenzoic acids, followed by oxidation of the corresponding intermediates **1a**,**b** with H_2O_2 in acetic acid (Scheme 1, for details see Online Supplementary Materials). To investigate the properties of modifiers under model conditions of binding to electrochemically inactive molecules, the corresponding *N*-isopropylbenzamides **2c**,**d** were prepared as comparative compounds by two alternative methods (see Scheme 1).

The ECR of modifiers **2a**,**b** in DMF represents an EEC-process (see Online Supplementary Materials). Contrary to ECR of the 2-methyl-9*H*-thioxanthen-9-one *S*,*S*-dioxide prototype,[31] dianions (DAs) of both acids are unstable, which leads to irreversibility of the second peak in their CV curves. The first step of the ECR for compounds **2a**,**b** represents a reversible one-electron transfer both in DMF and in DMF–H_2O mixtures, which is complicated by an additional peak probably originated from adsorption. The DMF replacement by its mixture with water leads to a shift in the half-wave potentials $E^1_{1/2}$ of the first ECR step towards less negative values. The $E^1_{1/2}$ values reveal good linear dependences in the range of H_2O molar fractions $0 < \chi < 0.46$. The changes in the $E^1_{1/2}$ potentials for compounds **2a** and **2b** are approximately equal, namely 0.1 and 0.09 V, respectively (see Online Supplementary Materials), and are lower than the corresponding values for fluorinated anthraquinones.[34]

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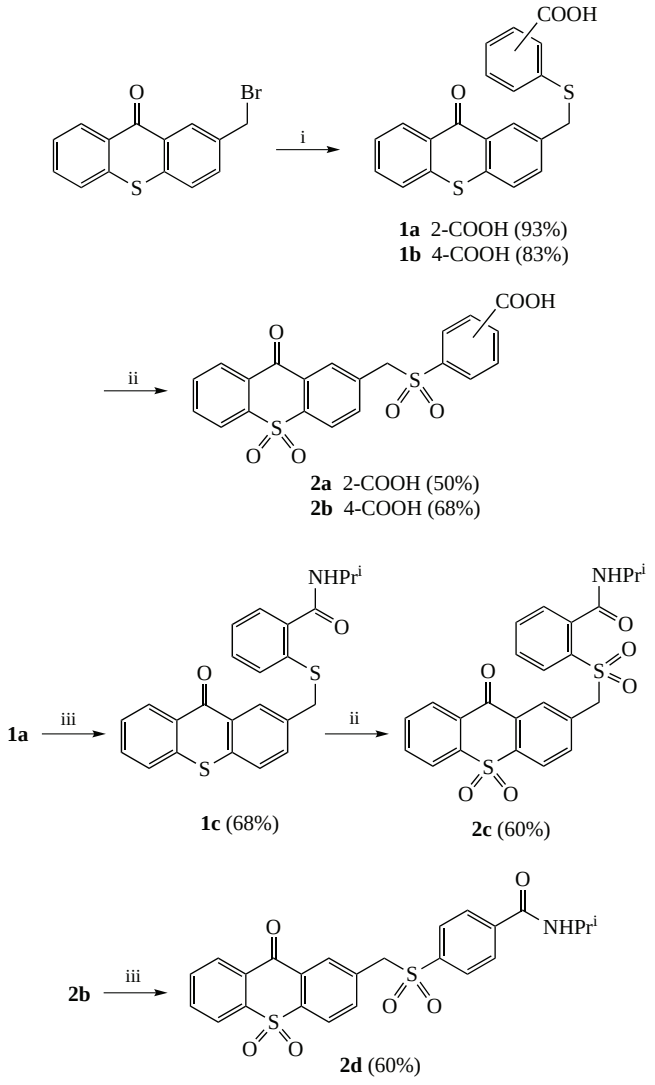

Scheme 1 *Reagents and conditions*: i, 2-HSC_6H_4COOH or 4-HSC_6H_4COOH, K_2CO_3, DMF, 80 °C; ii, H_2O_2, AcOH, reflux; iii, NHS, DCC, MeCN, 0 °C, then Pr^iNH_2, room temperature.

The CV curves for benzamides **2c**,**d** in DMF are associated with an EEC-process. The first step of their ECR represents one-electron transfer characterized by reversible CV waves in DMF and DMF–H_2O mixtures. The second irreversible ECR step reveals fast decay of the corresponding DAs (see Online Supplementary Materials).

Using the stationary ECR process for compounds **2a**–**d** in DMF and DMF–H_2O mixtures, EPR spectra of the corresponding radical anions (RAs) were obtained. The spectra revealed, that the unpaired electron was localized at the 9*H*-thioxantene-9-one *S*,*S*-dioxide moiety for all RAs of compounds **2a**–**d** (see Online Supplementary Materials). This is consistent with the results recently obtained for related RA of 2-[bis(4-aminophenyl)amino] methyl-9*H*-thioxanthen-9-one *S*,*S*-dioxide, which has been used as a monomer for the synthesis of electroactive polyimides.[35]

To examine acids **2a**,**b** as redox active modifiers for electrochemical detection of model DNA duplexes, the following oligonucleotides with complementary base sequences were prepared:

HS-$(CH_2)_6$-3′-CCGTCTTCGATACGCTATACCC-5′ oligoprobe 1
(**2a** or **2b**)-NH-$(CH_2)_6$-5′-GGCAGAAGCTATGCGATATGGG-3′ oligoprobe 2

Oligoprobe 1 was synthesized by standard automatic phosphoramidite synthesis, while oligoprobe 2 was obtained by modification of the corresponding amino-terminated 22-base oligonucleotide with succinimido derivatives of compounds **2a**,**b**. The sequence was chosen as a partial one from the quality control oligoprobe (QC) employed in microarray analysis.[36] A specially designed thin-layer electrochemical cell with three working electrodes equipped with a manual switch between them was used in the experiments. The surface of the working electrodes was cleaned in accordance with the procedure described[29] and then covered with microporous gold by electroplating to improve the detection sensitivity (see Online Supplementary Materials).

At first, all three working electrodes were modified with oligoprobe 1, which was immobilized on the surface using its HS anchor group [Figure 1(*a*)]. Then the working electrode no. 1 was subjected to hybridization with complementary oligoprobe 2. To determine the reference potential in the QC test, hybridization with a ferrocene-labeled oligonucleotide[29] was performed at the working electrode no. 2. Finally, no hybridization was made with the working electrode no. 3. Then washing was carried out using $(Et_3NH)HCO_3$ buffer (0.1 M, pH = 7.2) at room temperature in an automatic mode. The CV detection was performed in the same buffer, and results are presented in comparison with the data for benzoquinone- and naphthoquinone-labeled oligoprobes [Figure 1(*b*) and Online Supplementary Materials].[29]

The signal of **2b**-labeled DNA duplex proved to be reliable and was observed through 50–60 potential sweep cycles. Results for the duplex with label **2a** were quite similar. The assignment of the observed electrochemical responses to the 9*H*-thioxanthen-9-one *S*,*S*-dioxide moieties was confirmed by an independent experiment using compound **2d**.

In summary, we have synthesized two 9*H*-thioxanthen-9-one *S*,*S*-dioxide based redox active modifiers of oligonucleotides, which make possible an electrochemical detection of DNA

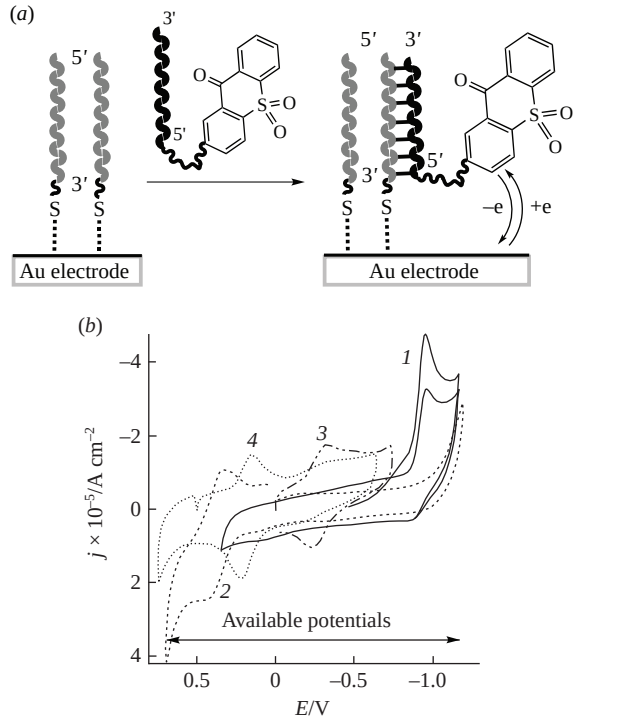

Figure 1 (*a*) Schematic representation of the DNA duplex formation using redox active labels **2a**,**b** at the 5′-end of oligoprobe 2, (*b*) CV detection of the DNA duplex formed by oligoprobe 1 immobilized on Au electrode surface with: (*1*) **2b**-labeled oligoprobe 2 (two cycles), (*2*) ferrocene-labeled oligoprobe used as a potential control, and CV detection of (*3*) naphthoquinone- and (*4*) benzoquinone-labeled oligoprobes.[29] CV curves were recalculated as current density *j* *vs*. potential at the potential sweep rate of 0.1 V s^{-1}.

duplexes on the surface of Au electrodes. Both redox active labels proved to be stable in their oligonucleotide and duplex structures and produced steady electrochemical output after hybridization with the complementary oligoprobe immobilized on Au electrode. The electrochemical response potential of the labels is well distinguished from the potentials of ferrocene-, benzo- and naphthoquinone based redox active labels,[29] and therefore these modifiers can be used in electrochemical genosensing, where a parallel detection of different DNA sequences with various redox active labels is required.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2020.05.011.

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