[9] The binding affinity is defined as $K_a = [\text{complex}]/([\text{sugar}][\text{cation}])$. NMR titrations were performed for **1**, **2**, and **3** at concentrations of 14, 17, and 10 mM in CD_3OD. About 100 equivalents of calcium chloride were added in portions and several well-separated chemical shifts were analysed. Experimental errors are below $\pm 3\,M^{-1}$. Compound **4** was investigated at 5 mM and at 20 mM, no significant differences were observed. Below temperatures of 250 K, severe signal broadening indicates higher aggregates of **4** in the presence of calcium chloride. The NMR titration of **4** in D_2O was performed at a concentration of 10 mM. Weak calcium affinities were observed for the hybrid oligosaccharide of lactose and Le^X ($K_a = 20\,M^{-1}$) and for a bistrisaccharide with the Le^X moieties anomerically linked by 1,3-propanediol through six rotatable bonds ($K_a = 17\,M^{-1}$).

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Solid-Phase Synthesis of Doubly Labeled Peptide Nucleic Acids as Probes for the Real-Time Detection of Hybridization**

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The mutual recognition of two complementary nucleic acid strands is the molecular basis for most of the current approaches in oligonucleotide-based diagnostics and therapy. Much work has therefore been devoted to the development of methods that allow for detection of the hybridization event. The hybridization assays that are most commonly used employ a solid phase in order to facilitate the separation of bound from unbound analytes.[1] In contrast, assays that proceed in homogeneous solution avoid problems such as nonspecific adsorption and the tedious washing protocols associated with heterogeneous assays.[2] In addition, homogeneous assays can provide a means to perform real-time measurements in vitro and even in vivo.[3] Conventional DNA-based probes, however, are susceptible to nucleolytic degradation which complicates their use in living cells. Herein, a method for real-time DNA measurements is presented that takes advantage of the enzyme-stable DNA-analogue peptide nucleic acid.[4] It is demonstrated that the application of a highly orthogonal protecting-group strategy, in combination with chemoselective conjugation reactions, allows for the rapid and automatable solid-phase synthesis of doubly labeled PNA probes suitable for homogeneous DNA detection.

Peptide nucleic acids (PNAs) bind with a remarkably high affinity and selectivity to complementary nucleic acids.[5] The structure of the resulting duplexes is similar to that of the corresponding DNA · DNA or DNA · RNA duplex molecules. However, there are striking differences between the structure of DNA and PNA single strands. For single-stranded PNA oligomers the analysis of the temperature-dependent UV absorbance reveals a sigmoidal melting curve.[6] The observed hypochromicity can reach values up to 30 %, which indicates that, in PNA single strands, base stacking is a favourable process. We reckoned that, due to this inter- or intramolecular association, a fluorescence-donor and a fluorescence-quencher group appended to the unhybridized PNA molecule **1** would be positioned in close proximity (Figure 1).[7] In analogy to the DNA-based molecular beacons **2**, which were introduced by the pioneering work of Kramer and Tyagi, collisional quenching and the distance-dependent fluorescence resonance energy transfer (FRET) would diminish the fluorescence of the donor label.[8a] Hybridization with complementary nucleic acids should induce a structural reorgan-

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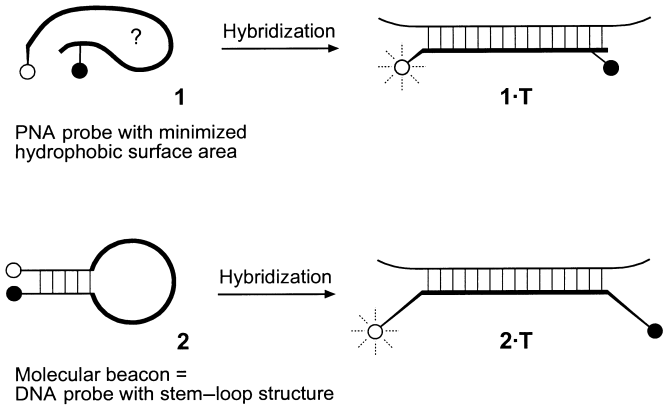
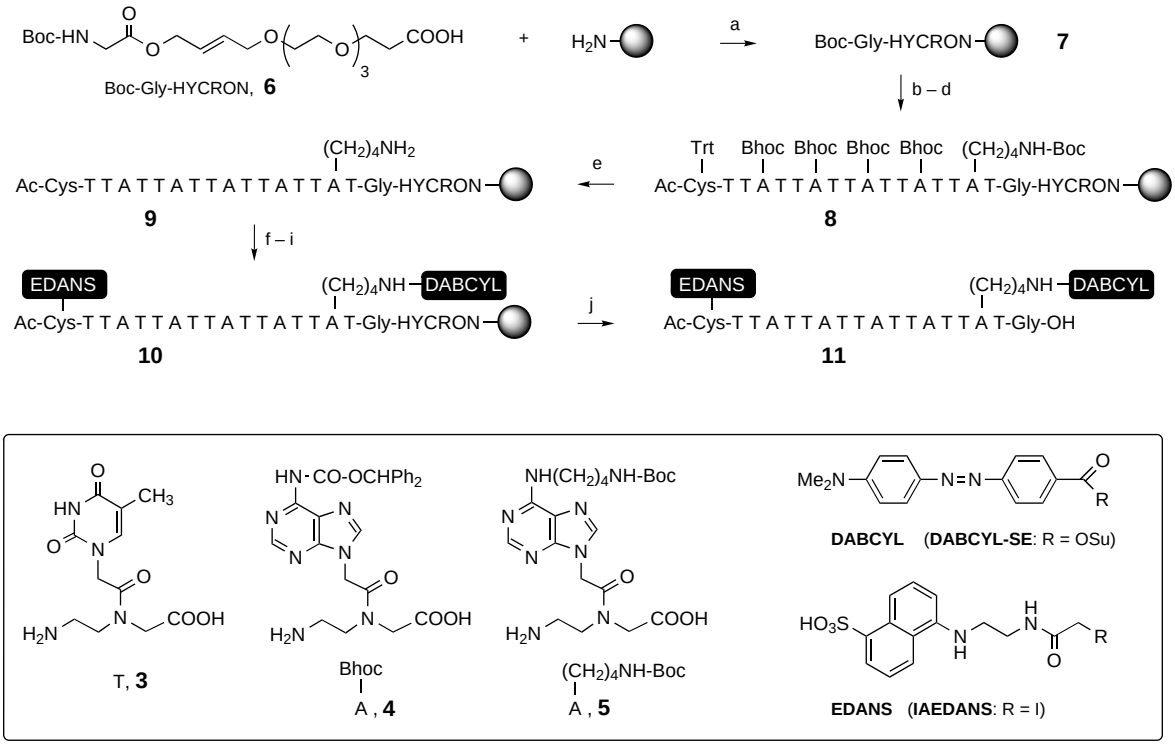


Figure 1. Unhybridized PNA forms intra- or intermolecular associates of unknown structure. The representation of the doubly labeled PNA probe **1** is intended to illustrate that appropriately appended fluorescence donor and fluorescence quencher groups could be located in close proximity. In analogy to the molecular beacons **2**, which are designed to form a stem–loop structure, the fluorescence is quenched due to collisional quenching and fluorescence resonance energy transfer (FRET). When the probe sequence anneals to the target sequence **T**, a structural reorganization increases the donor–quencher distance within the duplexes **1·T** and **2·T** and fluorescence can occur.

isation. In the double helical arrangement of duplexes **1·T** and **2·T** the distance between the donor and the quencher is increased and quenching processes become less likely to occur. As a result the doubly labeled probes become fluorescent upon hybridization.[8b,c] A remarkable feature of the PNA-based approach is that there is no need to incorporate secondary structure forming, but target-unrelated sequences.[9, 10]

For the synthesis of doubly labeled PNA conjugates, a highly flexible and automatable strategy was desired, which would enable all reactions, including the labeling steps, to be performed on the solid phase. Given the unknown structure of unhybridized PNA, it was unclear how the hybridization-induced change of the distance between the donor and the quencher group could be maximized. A strategy which combines terminal and internal labeling offers a high degree of flexibility. By shifting the internal conjugation site throughout the sequence, the donor–quencher distance can be varied without changing the length of the oligomer. Since access to the C terminus can be hindered on solid phases, the preferred strategy was to attach one label to the N terminus and the second to an internal conjugation site that would be provided by the N^6-aminoalkyl modified adenine building block **5**.[11] The HYCRON anchor was chosen as a linker.[12] Both acid- and base-labile protecting groups can be removed without detriment to the allylic ester linkage.[13]

The solid-phase synthesis of the PNA conjugates **11** and **12** proceeded as outlined in Scheme 1. *N*-Fluoren-9-ylmethoxycarbonyl (Fmoc) protected monomers were used with the benzhydryloxycarbonyl (Bhoc) and the *tert*-butyloxycarbonyl (Boc) groups employed for permanent protection of the exocyclic amino groups of the adenine **4** and the aminoalkyl tether of the N^6-modified adenine building block **5**, respec-



Scheme 1. a) 1. HBTU, HOBt, DIPEA, DMF; 2. Ac_2O:Pyr (1:10); b) 1. TFA; 2. DIPEA:DMF (1:9); c) iterative cycles of: 1. piperidine/DMF (1:4); 2. Fmoc-B^{Bhoc}-OH, HATU, $i Pr_2NEt$, Pyr, DMF; 3. Ac_2O, Pyr, DMF; d) 1. piperidine/DMF (1:4); 2. Ac_2O, Pyr, DMF; e) TFA:ethanedithiol:H_2O (95:2.5:2.5); f) DABCYL-SE (10 equiv), DMF:Pyr:NMM (7:1:1); g) piperidine/DMF (1:4); h) DTT (10 equiv), DMF:H_2O:NMM (9:3:1); i) IAEDANS (10 equiv), DMF:H_2O:NMM (9:3:1); j) [$Pd(PPh_3)_4$], morpholine, DMSO, DMF, 10% (based on **8**). DIPEA = *N*-Ethyl-*N*,*N*-diisopropylamine, DTT = dithiothreitol, HATU = *N*-[(dimethylamino)-1*H*-1,2,3-triazole[4,5-*b*]-pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate, HBTU = *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOBt = 1-hydroxy-benzotriazole, NMM = *N*-methylmorpholine.

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tively.[14] After completion of the iterative building-block coupling, the on-resin labeling was initiated. The *S*-trityl, the *N*-Boc, and all the Bhoc protecting groups were removed by treating the fully protected PNA **8** with a trifluoroacetic acid (TFA)/ethanedithiol/water mixture. The unprotected PNA oligomer remained attached to the HYCRON support.

The resin-bound PNA **9** was treated with the 4-(4′-dimethylaminophenylazo)benzoic acid hydroxysuccinimide ester (DABCYL-SE) in order to attach the DABCYL group to the primary amino group of the N^6-aminoalkyl modified adenine. Any thioester that would have been formed by a concomitant acylation of the cysteine thiol groups was cleaved by subsequent treatment with DMF/piperidine. Unprotected thiol groups, however, readily form disulfides. Hence, a dithiothreitol reduction was performed in order to liberate the thiol groups, to which the 5-(2-aminoethylamino) 1-naphthalene sulfonic acid (EDANS) group was attached by a selective alkylation with 5-(2′-iodoacetamidoethyl)amino-naphthalene sulfonic acid (IAEDANS).

The final detachment was accomplished by subjecting the resin-bound PNA conjugate **10** to a Pd^0-catalysed allyl transfer using the allyl scavenger morpholine. The subsequent reverse-phase HPLC purification furnished the PNA conjugate **11** and the positional isomer **12** in high purity. UV/Vis spectroscopy and matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) and nanospray mass analyses confirmed the identity of the doubly labeled PNA probes **11** and **12**.[15] It should be noted that, as the attachment of the EDANS and the DABCYL moieties conferred an increased hydrophobicity, the purification of the PNA conjugates was greatly facilitated.

It is known that the introduction of fluorescent labels affects the duplex stability, particularly when attached to internal nucleobases.[16] However, the PNA backbone confers a substantial stabilization which might be able to compensate for the perturbation introduced by the labeling. Indeed, analysis of the temperature-dependent UV absorbance revealed melting temperatures of 30.5 °C for the **11**·**13** duplex and 28.5 °C for the **12**·**13** duplex, which compare well with the temperature of 30.4 °C for the unmodified DNA·DNA duplex.[17]

Both conjugates, the doubly labeled PNA probes **11** and **12**, were used in hybridization experiments targeting the complementary oligonucleotide **13** and the sequence-unrelated oligonucleotide **14**. Figure 2 shows that the single-stranded PNA conjugates exhibited a quenched EDANS fluorescence. Addition of an excess of the mismatched oligonucleotide **14** led only to a minor change of the fluorescence spectrum (Figures 2 and 3). However, when a fourfold excess of the complementary oligonucleotide **13** was added at 25 °C, the fluorescence spectra changed dramatically. The emission of PNA probes **11** and **12** was enhanced by factors of 4.6 and 3, respectively. The intensification of the fluorescence was even more pronounced when the hybridizations were carried out at 20 °C, with enhancements by factors of 6.4 and 4.3, respectively. A spectral shift that could be indicative of a hydrophobicity-induced increase of the fluorescence was not detectable. It is thus likely that the mechanism by which the fluorescence increased followed the dequenching process that

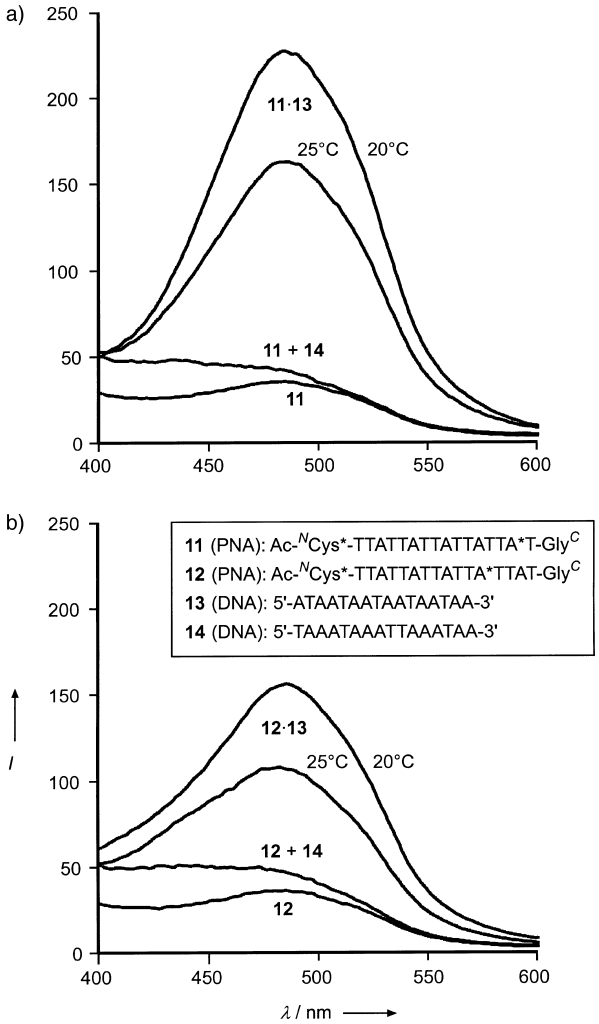

Figure 2. Normalized fluorescence spectra (arbitrary units) of a) the PNA probe **11** and b) the PNA probe **12**. The inset shows the oligomers used (Cys* = Cys(EDANS), A* = A(N^6(CH_2)$_4$NH-DABCYL)). Measurement conditions: 100 mM NaCl, 10 mM NaH_2PO_4, 0.1 mM EDTA, pH 7, 298 K, 1 μM **11** or **12**, 4 μM **13** or **14** when added, excitation at 335 nm. The uppermost curves were measured at 1.5 μM probe concentration and 293 K. EDTA = ethylenediaminetetraacetate.

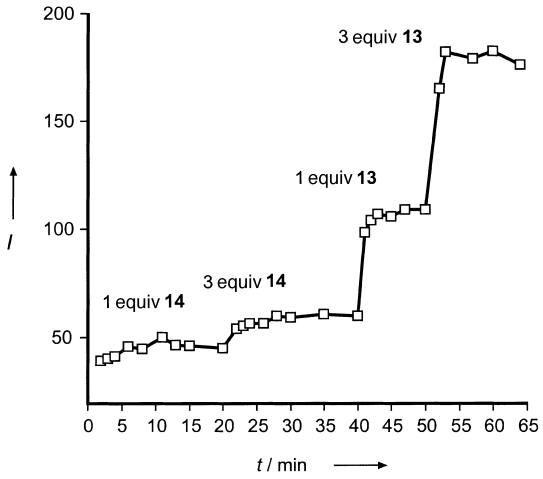

Figure 3. Fluorescence emission of probe **11** after addition of **14** (1 equiv, 0 min), **14** (3 equiv, 21 min), **13** (1 equiv, 40 min), and **13** (3 equiv, 51 min). Measurement conditions: 100 mM NaCl, 10 mM NaH_2PO_4, 0.1 mM EDTA, pH 7, 298 K, 1 μM of **11**, excitation at 335 nm.

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is observed for equally labeled molecular beacons. Analogous to molecular beacons, preparatory denaturation or renaturation procedures were not required and the probes began to fluoresce almost immediately after addition of the target oligonucleotide. As illustrated in Figure 3 the fluorescence of PNA probe **11** reached a plateau five minutes thereafter.

It was shown that the combined use of orthogonal protecting-group techniques and chemoselective conjugations allows for the rapid solid-phase synthesis of doubly labeled PNA probes. The PNA conjugates were demonstrated to be only weakly fluorescing in the single-stranded state. Hybridization of the weakly fluorescing PNA probes to a complementary oligonucleotide conferred a vivid fluorescence enhancement providing a means for homogeneous DNA detection. It has to be emphasized that target-unrelated arm sequences were not required for maintaining the structural integrity of the probes. The observed fluorescence quenching, however, might depend on both the sequence and the site of labeling. Future studies will reveal whether the fluorescence increase can be generalized to any sequence. Applications such as real-time polymerase chain reaction monitoring and real-time RNA detection in living cells could be feasible and benefit from the increased biostability of the PNA-based hybridization probes.

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Redox Switches with Chiroptical Signal Expression Based on Binaphthyl Boron Dipyrromethene Conjugates**

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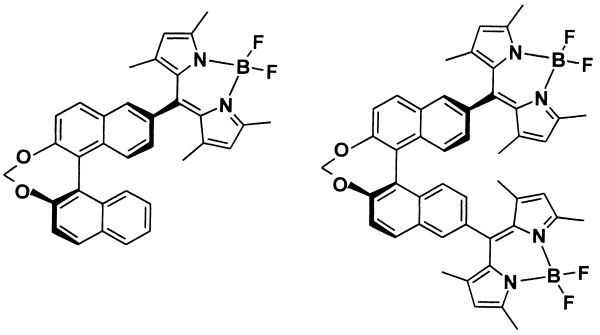
The interest in molecular building blocks with chiral and optoelectronic properties is driven on one hand by the higher specificity in molecular recognition and on the other by the polarization of electromagnetic radiation or light. Herein we report the synthesis and properties of optically active binaphthyl boron dipyrromethene (BDP) conjugates. Because of their favorable absorption and emission properties BDP dyes are frequently used as fluorescent probes for proton or metal ion detection,[1] in light-harvesting complexes within artificial photosynthetic arrays,[2] or as laser dyes.[3] It is also known that BDP dyes with appropriate functionalization can be reversibly oxidized and reduced.[1a, 4] Herein we present the optically active BDP derivatives (*R*)-**1** and (*R*)-**2** which were obtained by derivatization of a chiral 1,1′-



(*R*)-**1** (*R*)-**2**

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