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New cyanine dyes as base surrogates in PNA: Forced intercalation probes (FIT-probes) for homogeneous SNP detection

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Abstract—Forced intercalation probes (FIT-probes) are nucleic acid probes, in which an intercalator cyanine dye such as thiazole orange (TO) serves as a replacement of a canonical nucleobase. These probes signal hybridization by showing strong increases of fluorescence. TO in FIT-probes responds to adjacent base mismatches by attenuation of fluorescence intensities at conditions where both matched and mismatched target DNA are bound. The interesting features of TO labeled FIT-probes posed the question whether the forced intercalation concept can be extended to other cyanine dyes of the thiazole orange family. Herein, we present the synthesis of three asymmetrical cyanine dyes and their incorporation into PNA-conjugates by means of both divergent and linear solid-phase synthesis. Melting analysis revealed that the DNA affinity of PNA probes remained high irrespective of the replacement of a nucleobase by the cyanines YO (oxazole yellow), MO or JO. Of the three new tested dye–PNA-conjugates, the YO-containing PNA has properties useful for homogeneous SNP detection. YO–PNA is demonstrated to signal the presence of fully complementary DNA by up to 20-fold enhancement of fluorescence. In addition, YO emission discriminates against single base mismatches by attenuation of fluorescence. Oxazole yellow (YO) as a base surrogate in PNA may prove useful in the multiplex detection of single base mutations at non-stringent conditions.

1. Introduction

Recently, we introduced the concept of forced intercalation probes in homogeneous detection of single base mutations.[1–5] In these peptide nucleic acid (PNA)-based probes, a canonical nucleobase was replaced by an intercalator dye such as thiazole orange (TO, **1a**). After binding of FIT-probes to target DNA, the dye is forced to intercalate next to the envisioned mutation site (Fig. 1). We found that this mode of intercalation provided PNA-probes that responded to adjacent base mismatches by attenuation of fluorescence intensities.

Fluorescence-based discrimination between matched and single mismatched hybridization at non-stringent conditions has been reported for a variety of approaches including base-discriminating fluorescent nucleosides,[6–10] pyrene,[11–15] fluorene,[16] and fluorescein[17–20] modified nucleic acids, intercalator-quenched fluorescent probes,[21,22] and FRET-labeled ligation probes.[23,24] Also the use of phenanthridinium as an artificial DNA base has been demonstrated.[25,26] The FIT-PNA probes

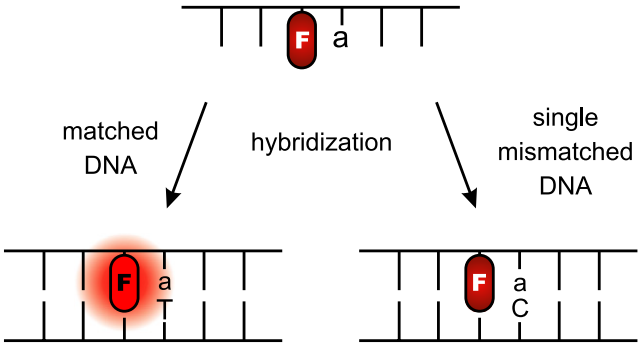

Figure 1. Design principle of FIT-probes. An intercalator fluorophore (F) serves as a base surrogate and is forced to intercalate adjacent to the expected mutation site. High fluorescence is only obtained upon formation of matched duplexes.

share features with another recently reported probe technology: Light-up probes.[27–30] Both are comprised of the fluorophore thiazole orange and PNA for recognition of DNA or RNA. However, thiazole orange in FIT-PNA is coupled as base surrogate, while the same dye in Light-up probes hangs via a flexible tether. It is this binding mode that confers the unique responsive-

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ness of TO fluorescence.[5] The property that distinguishes FIT-probes from Light-up probes and most other probes is the combination of the abilities to (1) signal hybridization by strong enhancements of fluorescence emission (up to 30-fold) and (2) distinguish matched from single mismatched hybridization at conditions where both matched and mismatched target DNA are bound.

The interesting results obtained with thiazole orange containing PNA posed the question whether other cyanine dyes of the thiazole orange family would show similar properties. Additional colors would find utility in multiplex assays, in which one color reports the presence of a specific DNA target, whereas the other color detects its single base mutant. We decided to explore forced intercalation of three additional asymmetrical cyanine dyes (**1b–d**) originally developed for unspecific DNA staining.[31–33] Here, we present full detail of the dye syntheses and the incorporation into PNA conjugates by linear and divergent solid-phase synthesis. The utility of the new FIT-probes in homogeneous SNP detection is evaluated.

2. Results

2.1. Dye synthesis

First, the cyanine dyes thiazole orange (TO in **1a**), oxazole yellow (YO in **1b**), thiazolopyridine (MO in **1c**), and oxazolopyridine (JO in **1d**) had to be equipped with carboxymethyl groups in order to allow attachment to PNA (Fig. 2). The required starting materials for the synthesis of thiazole orange derivative **1a** and oxazole yellow derivative **1b**, thio-benzothiazole (**6a**) and thio-benzoxazole (**6b**), are readily available. Thio-thiazolopyridine (**6c**) and thio-oxazolopyridine (**6d**) needed to be synthesized from 2-aminopyridine[34,35] (**3**) and 3-hydroxy-2-aminopyridine[35,36] (**2**), respectively (Scheme 1). First, **2** was converted to thio-oxazolopyridine (**6d**) upon treatment with potassium ethyl xanthate in refluxing EtOH. Thio-thiazolopyridine (**6c**) was synthesized by making use of ortho lithiation. 2-Aminopyridine (**3**) was allowed to react with pivaloyl chloride to provide pivalamide (**4**), which was treated sequentially with *n*-BuLi and tetraethylthiuram disulfide (TETD)[37] to yield 2-(pivalamido)pyridin-3-yl diethylcarbamodithioate (**5**).

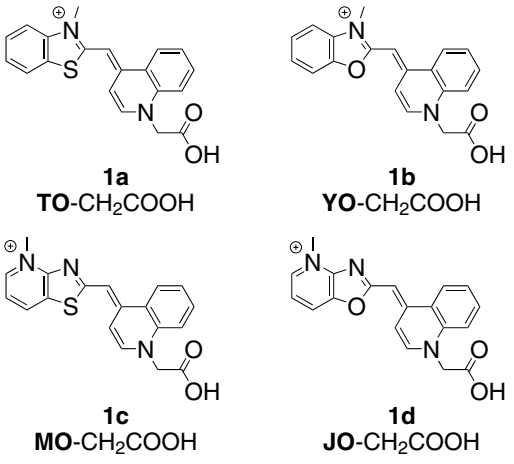
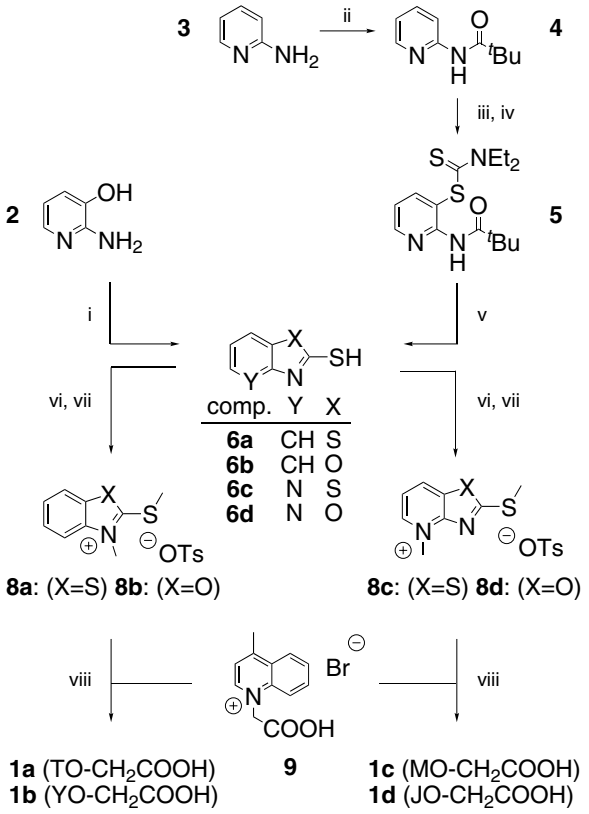


Figure 2. Structures of the synthesized cyanine dyes **1a–d**.

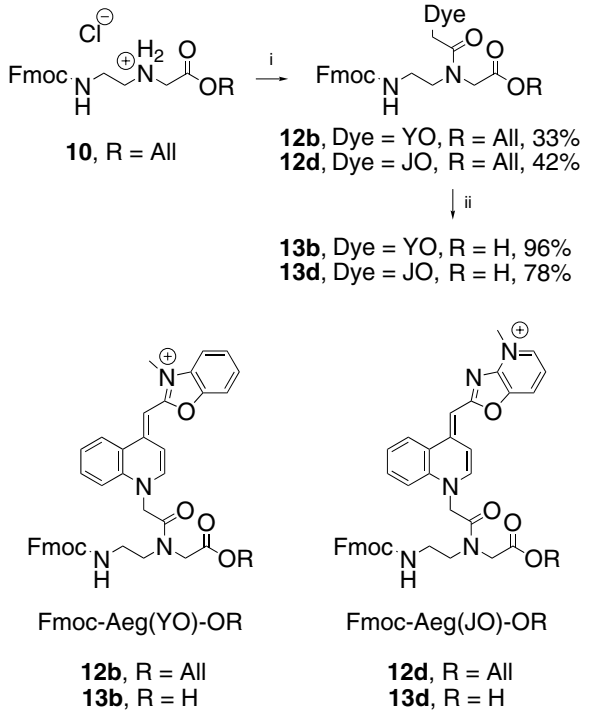


Scheme 1. Reagents and conditions: (i) EtOCSSK, EtOH, reflux, 41%; (ii) pivaloyl chloride, NEt_3, CH_2Cl_2, 0 °C, 94%; (iii) 2 equiv BuLi, THF, −50 °C; (iv) tetraethylthiuram disulfide (TETD), THF, −50 °C, 75%; (v) NaOH, MeOH, reflux, 37%; (vi) MeI, K_2CO_3, DMF, 0 °C, 72–96%; (vii) *p*-TsOMe, neat, 130 °C, 60–90%; (viii) 1-(carboxymethyl)-4-methyl-chinolinium bromide (**9**), NEt_3, CH_2Cl_2, 30–70%.

Finally, thio-thiazolopyridine (**6c**) was obtained by treatment of **5** with NaOH in refluxing MeOH. Conversion to the corresponding quaternary salts **8a–d** was performed by treating sequentially **6a–d** first with MeI in presence of K_2CO_3 in DMF to yield **7a–d**[35,36] and secondly with methyl *p*-toluenesulfonate.[38,39] The regioselectivity of quaternization was confirmed by HMBC NMR experiments. Quaternary thiazolium and oxazolium salts **8a–d** were then allowed to react with 1-carboxymethyl-4-chinolinium bromide (**9**) in the presence of NEt_3 to yield all four asymmetric cyanine dyes **1a–d**.[40]

2.2. Synthesis of PNA building blocks

The use of Fmoc/Bhoc-protected PNA monomers in automated solid-phase synthesis provides convenient access to thiazole orange labeled PNA-oligomers.[2] The required fluorescent Fmoc-PNA-monomers were prepared by coupling the asymmetric cyanine dyes TO (**1a**), YO (**1b**), and JO (**1c**) to the known Fmoc/allyl-protected aminoethylglycine backbone module **10** (Scheme 2).[41]



Scheme 2. Reagents and conditions: (i) 1b or 1d, PyBOP, PPTS, NMM, DMF, 16 h, 25 °C; (ii) $Pd(PPh_3)_4$, $PhNHCH_3$, THF, 16 h, 25 °C; [PyBOP, (benzotriazol-1-yloxy)-tripyrrolidinophosphonium hexafluorophosphat; PPTS, pyridinium *p*-toluenesulfonate; NMM, *N*-methylmorpholine].

The synthesis of the TO-containing monomer was previously described.[2] The rather challenging coupling reactions succeeded by using PyBOP as activation agent and PPTS to increase the low solubility of cyanine dyes.[42] Subsequent deprotection by Pd^0-catalyzed allyl transfer to *N*-methylaniline afforded the desired Fmoc-PNA-monomers (**13a**, **b**, **d**).

2.3. Solid-phase synthesis

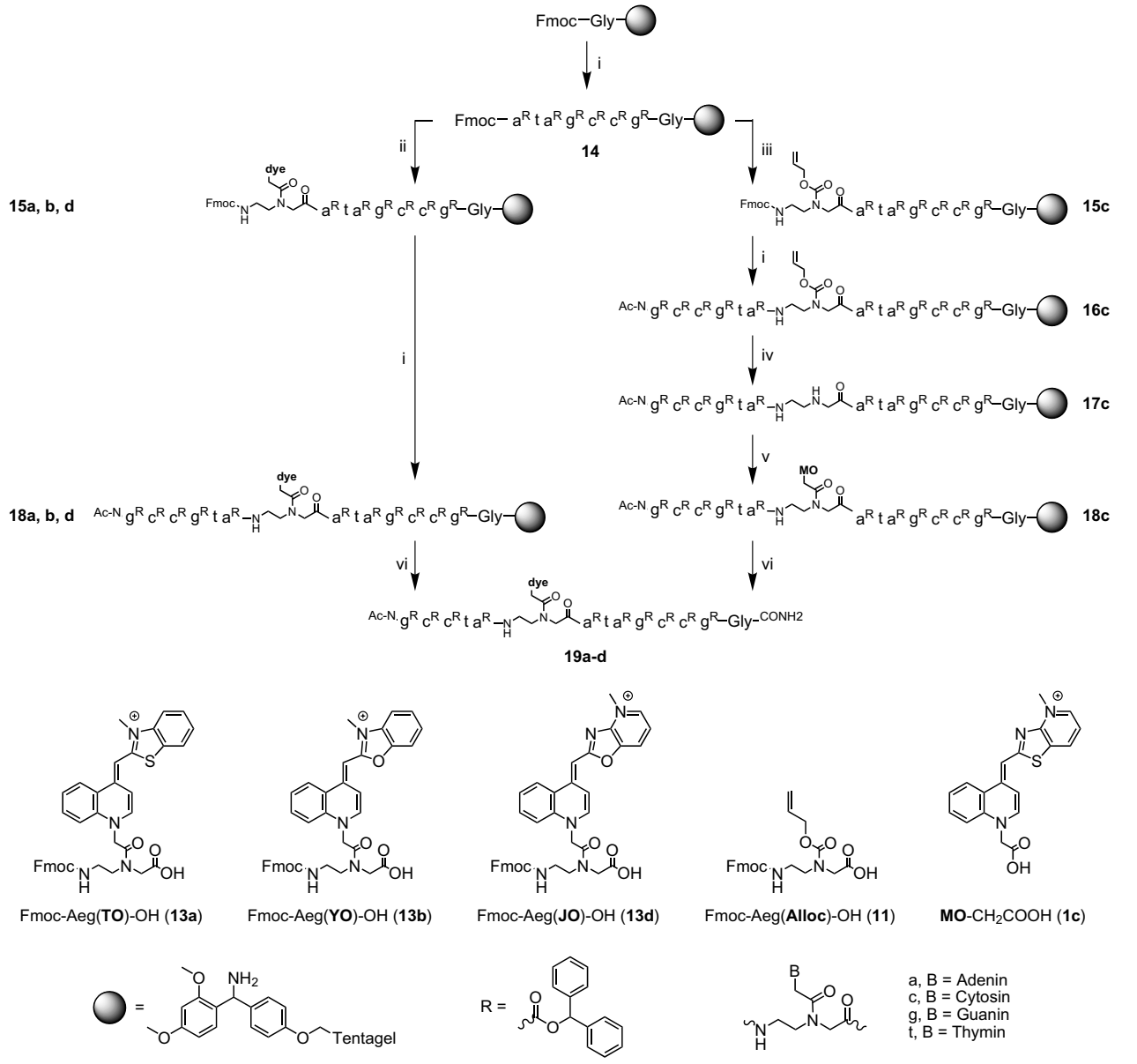
Fluorescent dye–PNA-conjugates were synthesized using both linear and divergent strategies.[2,5] While TO-, YO-, and JO-dyes were introduced as Fmoc-PNA-monomers, MO-dye labeling was achieved in a postsynthesis procedure. The fully protected PNA resin **14** was assembled first by using 4 equiv of commercially available Fmoc/Bhoc-protected PNA-monomers and 3.6 equiv of HCTU in presence of 6 equiv NMM using NMP as solvent (Scheme 3). Fluorescent Fmoc-PNA-monomers **13a**, **b**, **d** were introduced using double couplings to yield resins **15a**, **b**, **d**. In preparing for the on-resin introduction of the MO chromophore, backbone building block **11** was introduced (**15c**). Resins **15a–d** were extended to resins **18a**, **b**, **d** and **16c**. Orthogonally protected resin **16c** was treated with $Pd(PPh_3)_4$ in the presence of $BH_3{\cdot}NHMe_2$ to liberate the secondary amine in **17c**. The subsequent coupling with MO–CH_2COOH (**1c**) was accomplished by applying the previously optimized reagent combination PyBOP/PPTS to yield protected full-length resin **18c**.[2] For the release of conjugates **19a–d**, PNA resins **18a–d** were treated with TFA in the presence of *m*-cresol, water, and cysteine methyl ester as cation scavenger. The conjugates **19a–d** were purified by semi-preparative RP-HPLC. Analytical RP-HPLC, MALDI-TOF mass spectroscopy, UV/vis-, and fluorescence spectroscopy confirmed purity, molecular masses, and integrity of the chromophores (Table 1).

2.4. Hybridization experiments

The introduction of cyanine chromophores as base surrogates into the double helix inevitably leads to the omission of hydrogen bonding interactions. We have previously shown that thiazole orange can provide compensation for potential losses of duplex stability by means of its extraordinary base stacking ability.[4] Indeed, thiazole orange has been found to 'pair' to any of the four nucleobases with the strength of an AT base pair. To evaluate the ability of the three new dyes to maintain DNA affinity of the respective PNA-probes, melting curves were recorded and T_M values were determined (Table 2).

All cyanine-modified PNA–DNA duplexes showed sigmoid melting curves which suggested cooperative base pairing. The most stable duplex with complementary DNA **20** was formed by TO–PNA **19a**. YO–PNA **19b** closely resembles TO–PNA **19a** only differing by the oxazole which replaces the thiazole ring system of **19a**. The T_M of the YO–PNA–DNA duplex is lower by $\Delta T_M = 2$ °C than the T_M of the corresponding TO-containing duplex. This result suggests that TO better base stacks than YO, which is in line with the order of duplex affinities determined for free chromophores.[43] The comparison between MO- and JO-containing duplexes revealed, again, that the thiazole ring in MO–PNA **19c** conferred higher duplex stability than the oxazole ring in JO–PNA **19d** ($T_M = 71$ °C vs $T_M = 68$ °C). Nevertheless, it can be concluded that all studied cyanine base surrogates support the mutual recognition between FIT-PNA and DNA target. TO appears to provide the strongest stacking interactions, whereas JO probably causes the largest perturbation to the duplex.

The cyanine dyes YO, TO, JO, and MO have been selected because of their ability to signal binding to nucleic acids by enhancements of fluorescence.[31–33] We assumed that binding of PNA-conjugates **19b–d** to DNA **20** would also result in fluorescence enhancement as previously reported for TO–PNA **19a**. Figure 3 shows fluorescence spectra of PNA–dye-conjugates **19a–d** before (dashed lines) and after addition (solid lines) of complementary DNA **20**. As previously reported, addition of DNA **20** to TO–PNA **19a** led to strong increases of fluorescence by a factor of 19 at 25 °C. In contrast, YO–PNA **19b** responded to hybridization at room temperature by rather modest 4-fold fluorescence enhancements. Interestingly, fluorescence responsiveness of both TO- and YO–PNA **19a** and **19b**, respectively, was significantly higher at elevated temperature. For example, YO-containing duplex fluoresced at 60 °C with 18-fold higher intensity than single stranded **19b** (as opposed to only 4-fold at 25 °C, Table 3). More than



Scheme 3. Synthesis of dye–PNA-conjugates **19a**–**d**. Left side shows linear assembly strategy using fluorescent Fmoc-PNA-monomers **13a**, **b**, **d**. Right side shows divergent assembly strategy using orthogonally protected PNA-backbone module **11**. Subsequent deallylation and postassembly coupling of MO–CH_2COOH (**1c**) provide dye–PNA-conjugate **19c**. Reagents and conditions: (i) 1—piperidine, DMF (1:4); 2—Fmoc-B(Bhoc)-OH, NMM, HCTU, NMP; 3—Ac_2O, lutidine, DMF; (ii) 1—piperidine, DMF (1:4); 2—Fmoc-Aeg(dye)-OH (**13a**, **b**, **d**), NMM, HCTU, NMP; 3—Ac_2O, lutidine, DMF; (iii) 1—piperidine, DMF (1:4); 2—Fmoc-Aeg(Alloc)-OH (**11**), NMM, HCTU, NMP; 3—Ac_2O, lutidine, DMF; (iv) $Pd(PPh_3)\cdot BH_3$, $NHMe_2$, CH_2Cl_2; (v) MO–CH_2COOH (**1c**), PyBOP, PPTS, NMM, DMF; (vi) TFA, *m*-cresol, H_2O, HCysOMe.

Table 1. Synthesized PNA-conjugates **19a**–**d** and obtained overall yields after HPLC-purification

Compound	PNA-sequence	Yield
19a	$^{Ac\text{-}N}$gccga Aeg(TO) atagccgGly^{CONH_2}	3.4%
19b	$^{Ac\text{-}N}$gccga Aeg(YO) atagccgGly^{CONH_2}	7.2%
19c	$^{Ac\text{-}N}$gccga Aeg(MO) atagccgGly^{CONH_2}	1.8%
19d	$^{Ac\text{-}N}$gccga Aeg(JO) atagccgGly^{CONH_2}	7.2%

30-fold fluorescence intensification was obtained for hybridization of TO PNA **19a** at 60 °C. Cyanine–PNA-conjugates **19c** (MO) and **19d** (JO) proved less efficient in signaling of hybridization. Neither at 25 °C nor at 60 °C were fluorescence enhancements (up to 3-fold) as high as observed in TO- and YO–PNA **19a** and **19b** (Table 3).

In real-time PCR analysis, fluorescent probes bind to target DNA in the annealing phase between 50 and 70 °C. To evaluate the usefulness of the synthesized cyanine–PNA conjugates for real-time PCR applications, we examined the temperature dependence of fluorescence signaling. Figure 4 shows that the fluorescence enhancement F_{ds}/F_{ss} observed upon hybridization of TO–PNA **19a** reaches an optimum at elevated temperatures. More than 30-fold intensification of TO fluorescence is obtained when hybridization is performed at

Table 2. T_M values of PNA–DNA duplexes containing cyanine dyes as base surrogates

19a dye = TO; **19b** dye = YO; **19c** dye = MO; **19d** dye = JO

X	TO	YO	MO	JO
T_M (°C)	73	71	71	68

Measured as denaturation curves at 1 μM concentration in a buffered solution (100 mM NaCl, 10 mM NaH_2PO_4, pH 7).

55–70 °C as opposed to only 18-fold fluorescence enhancement at 25 °C. Relative fluorescence rapidly decreases at temperatures higher than T_M. The temperature-dependence of hybridization-induced fluorescence enhancement is even more pronounced for YO–PNA **19b**. For example, **19b** furnished only moderate 4-fold fluorescence enhancement at 25 °C, which is modest when compared to the 20-fold intensification at 70 °C. These results are important. They suggest that both TO- and YO-containing FIT-PNA may be useful at the typical temperatures applied in real time PCR analysis. In contrast, MO–PNA **19c** and JO–PNA **19d** will probably have limited utility due to their rather modest fluorescence responsiveness.

We next investigated the responsiveness of the chromophores in PNA-conjugates **19a** and **19b** toward adjacent base mismatches. MO–PNA **19c** and JO–PNA **19d** were not included in this study due to the insignificance of the hybridization-induced fluorescence changes. Fluorescence spectra of **19a** and **19b** were recorded before and after addition of single mismatched DNA **21A**, **G**, **C** and fluorescence enhancements F_{ds}/F_{ss} at specific wavelengths were determined (Fig. 5). Hybridization of single mismatched DNAs **21** resulted in all cases in lower fluorescence enhancements than hybridization with matched DNA **20**.

The background-corrected match/mismatch discrimination $D = (F_{ds}(\text{match}) - F_{ss})/(F_{ds}(\text{mismatch}) - F_{ss})$ is the important parameter in applications such as real-time PCR analysis. The previously demonstrated ability of

Table 3. Fluorescence properties of duplexes formed by dye–PNA-conjugates **19a–d** and complementary DNA **20**

	19a·20 (TO)	**19b·20** (YO)	**19c·20** (MO)	**19d·20** (JO)
F_{ds}/F_{ss} (25 °C)	19.4	4.3	1.9	0.9
F_{ds}/F_{ss} (60 °C)	31.0	18.5	2.7	2.3
λ (em, nm)	525	498	564	538

Measurement conditions as specified in Figure 3. F_{ds}/F_{ss} = fluorescence enhancement at λ (em) of dye–PNA conjugates **19a–c** upon hybridization to complementary DNA **20**. F_{ss} = fluorescence intensity of PNA single strand, F_{ds} = fluorescence intensity after addition of DNA **20**.

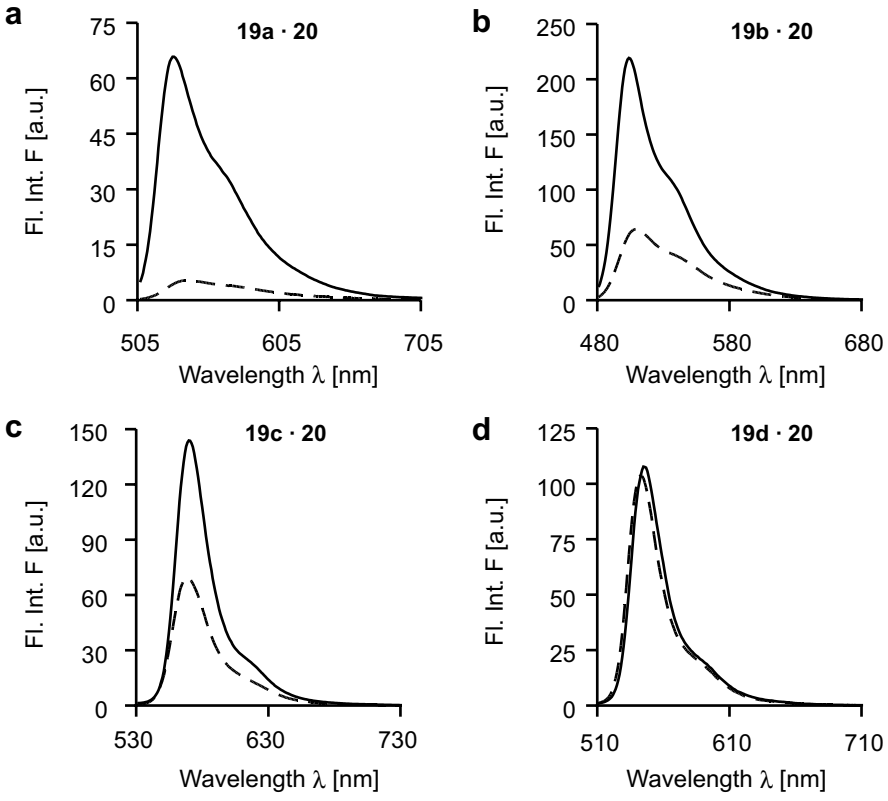


Figure 3. Fluorescence spectra of PNA-conjugates **19a–d**. Dashed lines: single stranded dye–PNA-conjugates, solid lines: PNA–DNA duplex. (a) **19a·20**,**19a**; (b) **19b·20**, **19b**; (c) **19c·20**, **19c**; (d) **19d·20**, **19d**. Measurement conditions: 1 μM dye–PNA-conjugate and DNA in buffer as specified in Table 2 at 25 °C. Excitation: (a) 495 nm; (b) 467 nm; (c) 517 nm; (d) 498 nm.

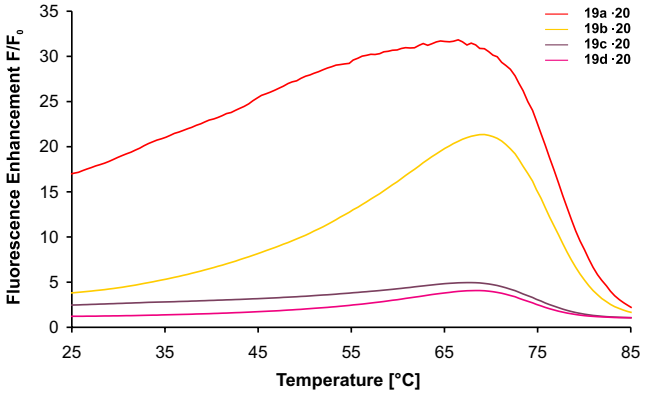


Figure 4. Temperature dependent fluorescence enhancement F_{ds}/F_{ss} upon hybridization of **19a–d** with complementary DNA **20**. Excitation **19a**: 515 nm, **19b**: 490 nm, **19c**: 560 nm, **19d**: 535 nm, Emission **19a**: 531 nm, **19b**: 504 nm, **19c**: 570 nm, **19d**: 545 nm.

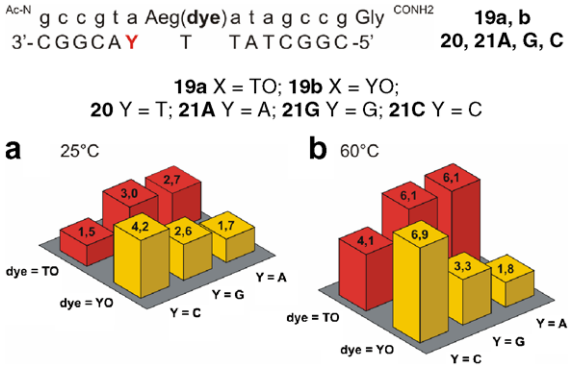


Figure 5. Background-corrected match/mismatch discrimination $D = (F_{ds}(\text{match}) - F_{ss})/(F_{ds}(\text{mismatch}) - F_{ss})$ of dye–PNA-conjugates **19a**,**b** after formation of match duplexes (Y = T) and mismatch duplexes (Y = C, G, A). (a) 25 °C; (b) 60 °C. F_{ss} = fluorescence intensity of PNA single strand, F_{ds} = fluorescence intensity of double strand formed after addition of DNA **21A**, **G**, **C** and **20**.

TO–PNA to discriminate matched from mismatched hybridization at non-stringent conditions became apparent also in this study. At 25 °C all matched and mismatched duplexes formed. However, probe **19a** was still able to discriminate the a-T match from a-A and a-G mismatches with 3-fold selectivity. Match/mismatch discrimination was lower ($D = 1.5$) for the a-C mismatch. Interestingly, this mismatch was easily discriminated ($D = 4.2$) by YO–PNA **19b**. In contrast, YO–PNA **19b** proved less suited ($D = 1.7$) to discriminate against the a-A mismatch. The single nucleotide specificity of fluorescence signaling can be improved by performing the hybridization at elevated temperature. At 60 °C, a temperature that was still below the T_M of mismatched probe-target complexes, match/mismatch discrimination increased to 4- to 6-fold for TO–PNA **19a** and up to 7-fold for YO–PNA **19b**. Further enhancements of sequence specificity are feasible at stringent conditions, at temperatures above the T_M of mismatched probe-target complexes (data not shown).

3. Conclusions

We have presented dye–PNA-conjugates in which the cyanine dyes thiazole orange (TO, **1a**), oxazole yellow (YO, **1b**), thiazolopyridine (MO, **1c**), and oxazolopyridine (JO, **1d**) served as replacement of a canonical nucleobase. The synthesis was performed by linear and divergent solid-phase synthesis. Melting analysis revealed that the introduction of cyanine base surrogates into PNA–DNA duplexes is tolerated. The DNA affinity of cyanine-modified PNA probes is not significantly affected. The obtained results provided evidence that the concept of forced intercalation probes (in which a fluorescent base surrogate is forced to intercalate next to the expected mutations site) is not restricted to the use of the previously explored thiazole orange dye. We found that PNA containing oxazole yellow (YO) as base surrogate is also suited to report the presence of fully complementary DNA by enhancements of fluorescence. In addition, YO emission responded to adjacent base mismatches by attenuated fluorescence emission at temperatures below the T_M. Both TO- and YO–PNA are hence suited to discriminate matched from single mismatched target at non-stringent hybridization conditions. Largest fluorescence enhancements are obtained when hybridization is performed at elevated temperatures typically applied in real-time PCR analysis. We wish to note that YO and TO fluorescence spectra can be resolved. It is thus possible to selectively excite/monitor YO fluorescence (e.g., at 490/504 nm) with only little crosstalk to TO fluorescence (515/531 nm). Future work will concern the combined use of FIT-YO- and FIT-TO–PNA in multiplexed real-time PCR analysis of single base mutations.

4. Experimental

4.1. General procedures and materials

Manual solid-phase synthesis (glycine-loading of resin) was performed by using 5 mL polyethylene syringe reactors that are equipped with a fritted disk. Automated linear solid-phase synthesis was performed by using an Intavis ResPep parallel synthesizer equipped with microscale columns for PNA synthesis. All column chromatography was performed with SDS 60 ACC silica gel using a Büchi Sepacore™ flash chromatographer and TLC with E. Merck Silica Gel 60 F254 plates. ^1H and ^{13}C NMR spectra were recorded with Bruker DPX 300 spectrometer. The signals of the residual protonated solvent ($CDCl_3$, CD_3OD or DMSO-d_6) were used as reference signals. Coupling constants are given in Hertz. HPLC was performed with an Agilent 1100 series instrument using a Varian Polaris-C18 A 5μ (PN A 2000-250x046) at 55 °C for analytical runs and a Varian Polaris C18 A 5μ (PN A 2000-250-100) for semipreparative runs. Eluents analytical: A (0.1% HCOOH in water + 1% MeCN) and B (0.1% HCOOH in MeCN + 1% water); semipreparative: A (0.1% TFA in water + 1% MeCN) and B (0.1% TFA in MeCN + 1% water) were used in a linear gradient with a flow rate of 1 mL/min for analytical and 6 mL/min for

semipreparative HPLC. High-resolution mass spectra were measured with a Hewlett-Packard GCMS 5995-A (ESI+) spectrometer. MALDI-TOF mass spectra were recorded with a Voyager-DE™ Pro Biospectrometry Workstation of PerSeptive Biosystems. DNA was purchased from MWG-Biotech in HPSF quality. Fmoc/Bhoc-protected PNA monomers were purchased from Applied Biosystems. Water was purified with a Milli-Q® Ultra Pure Water Purification System (Millipore Corp.). Solvents were dried and distilled following standard procedures if needed. Dry DMF ($H_2O < 0.01\%$) was purchased from Fluka. All other used compounds were commercially available and not further purified prior to usage.

4.2. Fluorescence spectrometry

Fluorescence spectra were recorded by using a Varian Cary Eclipse spectrometer. Measurements were carried out in fluorescence quartz cuvettes (4× 10 mm) at 1 μM concentration in a buffered solution (100 mM NaCl, 10 mM NaH_2PO_4, pH 7). Excitation: TO: 495 nm; YO: 467 nm; MO: 517 nm; JO: 498 nm; Slit$_{Ex}$ = 5, Slit$_{Em}$ = 2.5. After addition of DNA and dye–PNA-conjugates, solutions were heated to 95 °C before cooling to 25 or 60 °C. Spectra were recorded after 5 min at 25 or 60 °C, thus obtaining constant emission.

4.2.1. Carboxymethylated thiazole orange (1a). To a solution of 2.91 g (7.92 mmol) 3-methyl-2-(methylthio)-benzothiazolium tosylate (**8a**) and 2.79 g (9.90 mmol) 1-(carboxymethyl)-4-methyl-chinolinium bromide (**9**) in 150 mL CH_2Cl_2 was added 2.00 g (19.8 mmol, 2.78 mL) triethylamine, which caused immediate change of color to red. The mixture was stirred under exclusion of light at room temperature for 16 h. All volatiles were removed under reduced pressure, the residue was dissolved in 600 mL refluxing MeOH, 1.5 L H_2O was added, and the resulting solution was stored at 4 °C over 3 days. The resulting precipitate was collected by filtration and dried under reduced pressure to yield **1a** as a red solid (1.83 g, 54 %). TLC $R_f = 0.37$ ($CHCl_3$/MeOH/AcOH 5:3:2). ^{1}H NMR (DMSO-d_6/TFA) δ ppm: 4.02 (3H, s, CH_3), 5.45 (2H, s, CH_2), 6.93 (1H, s, CH), 7.32 (1H, d, J = 7.3, ArH), 7.42 (1H, t, J = 7.6, ArH), 7.60 (1H, t, J = 7.5, ArH), 7.69 (1H, t, J = 7.7, ArH), 7.78 (1H, d, J = 8.4, ArH), 7.83 (1H, d, J = 8.6, ArH), 7.92 (1H, t, J = 8.4, ArH), 8.03 (1H, d, J = 7.7, ArH), 8.49 (1H, d, J = 7.3, ArH), 8.75 (1H, d, J = 8.4, ArH). ^{13}C NMR (DMSO-d_6/TFA) δ ppm: 33.9 (CH_3), 54.6 (CH_2), 88.8 (CH), 107.4, 113.2, 117.64, 122.8 (4× ArC), 123.6, 124.1 (2× ArC_q), 124.7, 125.6, 126.6, 128.2, 133.2 (5× ArC), 137.7, 140.3 (2× ArC_q), 144.8 (ArC), 148.5, 160.8, 168.7 (3× ArC_q). HR-MS: m/z calcd for $C_{20}H_{17}N_2O_2S^+$ 349.1005, found 349.1005 $[M]^+$.

4.2.2. Carboxymethylated oxazole yellow (1b). Following the procedure of **1a** were 5.00 g (14.2 mmol) 3-methyl-2-(methylthio)-benzoxazolium tosylate (**8b**), 5.00 g (17.7 mmol) 1-(carboxymethyl)-4-methyl-chinolinium bromide (**9**), and 2.88 g (28.5 mmol, 4.00 mL) triethylamine in 260 mL CH_2Cl_2 allowed to react to yield **1b** as a pale red solid (2.18 g, 37 %). TLC $R_f = 0.35$ ($CHCl_3$/MeOH/AcOH 5:3:2). ^{1}H NMR (DMSO-d_6/TFA) δ ppm: 3.87 (3H, s, CH_3), 5.45 (2H, s, CH_2), 6.30 (1H, s, CH), 7.39 (1H, d, J = 7.6, ArH), 7.47 (1H, d, J = 7.6, ArH), 7.66 (2H, m, 2ArH), 7.80 (2H, m, 2ArH), 7.90 (2H, m, 2ArH), 8.37 (1H, d, J = 7.4, ArH), 8.74 (1H, d, J = 7.4, ArH). ^{13}C NMR (DMSO-d_6/TFA) δ ppm: 30.6 (CH_3), 54.4 (CH_2), 74.8 (CH), 108.6, 110.8, 110.9, 117.5 (4ArC), 122.8 (ArC_q), 124.5, 125.9, 125.9, 126.3 (4× ArC), 131.2 (ArC_q), 133.2 (ArC), 137.8 (ArC_q), 144.0 (ArC), 146.0, 150.1, 161.6, 168.7 (4× ArC_q). HR-MS: m/z cacld for $C_{20}H_{17}N_2O_3^+$ 333.1234, found 333.1231 $[M]^+$.

4.2.3. Carboxymethylated thiazolopyridine (1c). Following the procedure of **1a** were 875 mg (2.37 mmol) 4-methyl-2-methylthio-thiazolopyridinium tosylate (**7c**), 670 mg (2.37 mmol) 1-(carboxymethyl)-4-methyl-chinolinium bromide (**9**), and 480 mg (4.74 mmol, 659 μL) triethylamine in 50 mL CH_2Cl_2 allowed to react. The crude product was recrystallized from MeOH to yield **1c** as a rusty brown solid (305 mg, 30 %). ^{1}H NMR (DMSO-d_6/TFA) δ ppm: 4.21 (3H, s, CH_3), 5.28 (2H, s, CH_2), 6.84 (1H, s, CH), 7.14 (1H, dd, J_1 = 6.4, J_2 = 7.6, ArH), 7.46 (1H, m, ArH), 7.57 (1H, d, J = 8.3, ArH), 7.73 (1H, m, ArH), 8.01 (1H, d, J = 7.5, ArH), 8.35 (4H, m, 4ArH). ^{13}C NMR (DMSO-d_6/TFA) δ ppm: 42.6 (CH_3), 54.0 (CH_2), 94.3 (CH), 109.1, 115.6, 116.8 (3× ArC), 122.6 (ArC_q), 124.9, 125.5 (2× ArC), 131.7 (ArC_q), 132.2, 133.9 (2× ArC), 138.0 (ArC_q), 142.6, 146.4 (2× ArC), 146.4, 158.4 (2× ArC_q), 169.1 (Cq), 172.7 (ArC_q). HR-MS: m/z calcd for $C_{19}H_{16}N_3O_2S^+$ 350.0958, found 350.0961 $[M]^+$.

4.2.4. Carboxymethylated oxazolopyridine (1d). Following the procedure of **1a** were 3.19 g (9.05 mmol) 4-methyl-2-(methylthio)-oxazolopyridinium tosylate (**8d**), 2.56 g (9.05 mmol) 1-(carboxymethyl)-4-methyl-chinolinium bromide (**9**), and 1.83 g (18.1 mmol, 2.54 mL) triethylamine in 150 mL CH_2Cl_2 allowed to react. The crude product was recrystallized from MeOH to yield **1d** as a violet solid (2.61 g, 70%). TLC $R_f = 0.29$ ($CHCl_3$/MeOH/AcOH 5:3:2). ^{1}H NMR (DMSO-d_6/TFA) δ ppm: 4.12 (3H, s, CH_3), 5.34 (2H, s, CH_2), 6.47 (1H, s, CH), 7.31 (1H, t, J = 7.1, ArH), 7.55 (1H, t, J = 7.6, ArH), 7.68 (1H, d, J = 8.6, ArH), 7.82 (1H, t, J = 7.7, ArH), 8.19 (4H, m, 4ArH), 8.46 (1H, d, J = 8.4 Hz, ArH). ^{13}C NMR (DMSO-d_6/TFA) δ ppm: 40.3 (CH_3), 54.0 (CH_2), 81.8 (CH), 108.5, 116.3, 117.1, 118.6 (4× ArC), 122.8 (ArC_q), 125.0, 125.8, 132.6, 135.9 (ArC), 137.7 (ArC_q), 142.6 (ArC), 145.1, 149.9, 153.9 (3× ArC_q), 169.0 (C_q), 171.4 (ArC_q). HR-MS: m/z calcd for $C_{19}H_{16}N_3O_3^+$ 334.1186, found 334.1186 $[M]^+$.

4.2.5. 2-(Pivalamido)pyridine (4). To a solution of 4.71 g (50.0 mmol) 2-aminopyridine (**3**) and 6.35 g (62.5 mmol, 8.78 mL) triethylamine in 100 mL CH_2Cl_2 at 0 °C was added a solution of 6.63 g (55.0 mmol) pivaloyl chloride in 50 mL CH_2Cl_2 dropwise over 2 h. After stirring for further 2 h, the reaction mixture was allowed to warm to room temperature and stirred further for 60 min. The reaction was then quenched by addition of

100 mL H_2O. The organic layer was separated, washed with 50 mL sat. $NaHCO_3$ solution (2×), dried ($MgSO_4$), and evaporated. The residue was recrystallized from cyclohexane to yield **4** as a white crystalline solid (8.38 g, 94 %). TLC $R_f = 0.42$ (cyclohexane/EtOAc 2:1). ^1H NMR ($CDCl_3$) δ ppm: 1.31 (9H, s, $3CH_3$), 7.02 (H, m, ArH), 7.69 (1H, m, ArH), 8.12 (1H, s, NH), 8.24 (2H, m, ArH). ^{13}C NMR ($CDCl_3$) δ ppm: 27.4 ($3CH_3$), 39.8 (C_q), 113.9, 119.6, 138.5, 147.3 (4× ArC), 151.4 (ArC_q), 177.1 (C_q).

4.2.6. 2-(pivalamido)pyridine-3-yl-diethylcarbamodithioate (5). To a solution of 8.32 g (46.7 mmol) 2-(pivalamido)pyridine (**4**) in 100 mL THF at −50 °C was added *n*-BuLi (2.5 M in hexane, 39.2 mL, 98.0 mmol) in two equimolar portions dropwise over 5 min each. After the addition was complete, the yellowish solution was stirred for 30 min at −50 °C, warmed to 0 °C, and stirred for another 4 h at which time a yellow precipitate appeared. The reaction mixture was cooled to −50 °C, a solution of 16.6 g (56.0 mmol) tetraethylthiuram disulfide (TETD) in 50 mL THF was added dropwise, and the resulting mixture stirred for 30 min. Cooling was abandoned and after stirring overnight 250 mL Et_2O was added. The organic phase was washed with 300 mL H_2O (2×) and the combined aqueous phases were reextracted with 200 mL Et_2O. The combined organic phases were dried ($MgSO_4$), evaporated, and purified by flash column chromatography (100 % cyclohexane → 100 % EtOAc) to yield **5** as a white solid (11.5 g, 75%). TLC $R_f = 0.48$ (EtOAc). ^1H NMR ($CDCl_3$): δ ppm: 1.27 (12H, m, $4CH_3$), 1.38 (3H, t, $J = 7.1$, CH_3), 3.84 (2H, q, $J = 7.1$, CH_2), 4.00 (2H, q, $J = 7.1$, CH_2), 7.14 (1H, dd, $J_1 = 4.8$, $J_2 = 7.7$, ArH), 7.76 (1H, dd, $J_1 = 1.8$, $J_2 = 7.7$, ArH), 8.52 (1H, s, NH), 8.60 (1H, dd, $J_1 = 1.8$, $J_2 = 4.8$, ArH). ^{13}C NMR ($CDCl_3$) δ ppm: 11.4, 12.7 (2× CH_3), 27.3 ($3CH_3$) 39.7 (C_q), 47.6, 49.9 (2× CH_2), 120.4 (ArC_q), 120.9, 145.7, 150.7 (3× ArC), 153.8 (ArC_q), 176.2, 188.5 (2× C_q).

4.2.7. 2-Thio-thiazolopyridine (6c). A solution of 1.54 g (4.73 mmol) 2-(pivalamido)pyridine-3-yl-diethyl-carbamodithioate (**5**) and 5.85 g (147 mmol) NaOH in 30 mL EtOH was refluxed over 4 h. After cooling to room temperature, 75 mL of 2 M hydrochloric acid was added whereupon an off-white precipitate appeared. The precipitate was collected by filtration, washed with H_2O, and dried under reduced pressure to yield **6c** as an off-white solid (340 mg, 43%). TLC $R_f = 0.68$ (EtOAc). ^1H NMR (DMSO-d_6) δ ppm: 7.30 (1H, dd, $J_1 = 4.9$, $J_2 = 7.9$, ArH), 8.13 (1H, dd, $J_1 = 1.5$, $J_2 = 7.9$, ArH), 8.36 (1H, dd, $J_1 = 1.5$, $J_2 = 4.9$, ArH), 14.30 (1H, s, SH). ^{13}C NMR (DMSO-d_6) δ ppm: 119.5 (ArC), 124.1 (ArC_q), 130.5, 146.7 (2× ArC), 153.7, 190.9 (2× ArC_q).

4.2.8. 2-Thio-oxazolopyridine (6d). To a solution of 5.00 g (45.5 mmol) 2-amino-3-hydroxy-pyridine (**2**) in 100 mL EtOH (abs) under an atmosphere of Ar was added 15.0 g (93.8 mmol) potassium ethyl xanthate in 5 equimolar portions every 3 h. After cooling, volatiles were removed under reduced pressure, the residue dissolved in H_2O and acidified to pH 5 with glacial acetic acid. The resulting precipitate was collected by filtration, washed with H_2O, and dried under reduced pressure to yield **6d** as a brownish solid (2.85 g, 41%). ^1H NMR (DMSO-d_6) δ ppm: 3.37 (1H, br, NH), 7.27 (1H, dd, $J_1 = 5.2$, $J_2 = 8.1$, ArH), 7.87 (1H, dd, $J_1 = 8.1$, $J_2 = 1.3$, ArH), 8.22 (1H, dd, $J_1 = 5.2$, $J_2 = 1.2$, ArH). ^{13}C NMR (DMSO-d_6) δ ppm: 117.0, 119.1 (2× ArC), 141.6 (ArC_q), 144.1 (ArC), 146.9, 181.3 (2× ArC_q).

4.2.9. 2-(Methylthio)-benzothiazole (7a). To a solution of 16.7 g (100 mmol) 2-thiobenzothiazol (**6a**) in 250 mL DMF was added 13.8 g (100 mmol) K_2CO_3 and stirred over 15 min. To this mixture was added 16.9 g (120 mmol, 7.42 mL) MeI in one portion. After stirring for 1 h of 500 mL H_2O was added. The resulting precipitate was collected by filtration and dried under reduced pressure to yield **7a** as a white solid (16.3 g, 90%). ^1H NMR (CD_3OD) δ ppm: 2.70 (3H, s, CH_3), 7.24 (1H, t, $J = 7.7$, ArH), 7.36 (1H, t, $J = 7.7$, ArH), 7.74 (2H, m, 2ArH). ^{13}C NMR (CD_3OD) δ ppm: 16.1 (S–CH_3), 122.0, 122.3, 125.4, 127.4 (4× ArC), 136.1, 154.4, 170.7 (3× ArC_q).

4.2.10. 2-(Methylthio)-benzoxazole (7b). To a solution of 10.0 g (66.1 mmol) 2-thiobenzoxazole (**6b**) in 200 mL DMF was added 139.1 g (66.1 mmol) K_2CO_3 and stirred over 15 min. To this mixture was added 10.3 g (79.3 mmol, 4.52 mL) MeI in one portion. After stirring for 1 h of 600 mL H_2O was added. The reaction mixture was extracted with 200 mL EtOAc (3×). The combined organic phases were washed with 100 mL H_2O (3×), brine (1×), dried ($MgSO_4$), evaporated, and dried under reduced pressure to yield **7b** as a brown oil (9.53 g, 87%). TLC $R_f = 0.58$ (cyclohexane/EtOAc 10:1). ^1H NMR ($CDCl_3$) δ ppm: 2.78 (3H, s, CH_3), 7.25 (1H, t, $J = 7.2$, ArH), 7.30 (1H, t, $J = 7.1$, ArH), 7.46 (1H, d, $J = 7.8$, ArH), 7.63 (1H, d, $J = 7.6$, ArH). ^{13}C NMR ($CDCl_3$) δ ppm: 14.5 (S–CH_3), 109.8, 118.3, 123.7, 124.2 (4× ArC), 141.9, 151.9, 165.7 (3× ArC_q).

4.2.11. 2-Methylthio-thiazolopyridine (7c). With the experimental setup described for **7b** were 1.61 g (9.55 mmol) 2-thio-thiazolopyridine (**6c**), 1.32 g (9.55 mmol) K_2CO_3, and 1.49 g (10.5 mmol, 654 μL) MeI in 100 mL DMF allowed to react to yield **7c** as an off-white solid (1.66 g, 96%). TLC $R_f = 0.64$ (EtOAc). ^1H NMR (CD_3OD) δ ppm: 2.80 (3H, s, S–CH_3), 7.30 (1H, dd, $J_1 = 4.8$, $J_2 = 8.0$, ArH), 8.32 (1H, dd, $J_1 = 1.5$, $J_2 = 8.0$, ArH), 8.48 (1H, dd, $J_1 = 1.4$, $J_2 = 4.8$, ArH). ^{13}C NMR (CD_3OD) δ ppm: 16.0 (S–CH_3), 120.5 (ArC), 130.5 (ArC_q), 132.4, 148.2 (2× ArC), 164.7, 176.1 (2× ArC_q).

4.2.12. 2-(Methylthio)-oxazolopyridine (7d). With the experimental setup described for **7b** were 2.50 g (16.4 mmol) 2-thio-oxazolopyridine (**6d**), 2.26 g (16.4 mmol) K_2CO_3, and 2.80 g (19.7 mmol, 1.23 mL) MeI in 40 mL DMF allowed to react to yield **7d** as a white solid (1.97 g, 72%). ^1H NMR ($CDCl_3$) δ ppm: 2.78 (3H, s, S–CH_3), 7.16 (1H, dd, $J_1 = 5.0$, $J_2 = 8.1$, ArH), 7.68 (1H, dd, $J_1 = 8.0$, $J_2 = 1.5$, ArH), 8.43 (1H, dd, $J_1 = 5.0$, $J_2 = 1.3$, ArH). ^{13}C NMR ($CDCl_3$) δ

ppm: 14.7 (S–CH_3), 117.2, 118.8 (2× ArC), 144.2 (ArC_q), 145.5 (ArC), 155.9, 170.5 (ArC_q).

4.2.13. 3-Methyl-2-(methylthio)-benzothiazolium tosylate (8a). To 5.82 g (32.1 mmol) 2-methylthiobenzothiazole (**7a**) was added 6.58 g (35.3 mmol, 5.33 mL) methyl *p*-toluenesulfonate and heated to 130 °C for 1 h. After cooling to 70 °C, acetone was added until a white precipitate appeared. Reflux was maintained for another 30 min before cooling to room temperature. The precipitate was collected by filtration and dried under reduced pressure to yield **8a** as a pale yellow solid. (9.98 g, 85%). ^{1}H NMR (CD_3OD) δ ppm: 2.29 (3H, s, CH_3), 3.06 (3H, s, S–CH_3), 4.08 (3H, s, N^+–CH_3), 7.13 (2H, d, J = 8.1, 2ArH), 7.61 (2H, d, J = 8.2, ArH), 7.67 (1H, t, J = 7.7, ArH), 7.79 (1H, t, J = 7.5, ArH), 8.01 (1H, d, J = 8.5, ArH), 8.16 (1H, d, J = 8.1, ArH). ^{13}C NMR (CD_3OD) δ ppm: 18.5 (S–CH_3), 21.4 (CH_3), 36.9 (N^+–CH_3), 116.5, 124.7 (2× ArH), 126.9 (2ArH), 128.5 (ArH), 129.8 (2ArH), 129.9 (ArC_q), 130.7 (ArH), 141.6, 143.7, 144.1, 183.2 (4× ArC_q).

4.2.14. 3-Methyl-2-(methylthio)-benzoxazolium tosylate (8b). To 9.53 g (57.7 mmol) 2-methylthiobenzoxazole (**7b**) was added 19.6 g (105 mmol, 15.9 mL) methyl *p*-toluenesulfonate and heated to 130 °C for 1 h. After cooling to 70 °C, cyclohexane was added. Reflux was maintained for another 30 min before cooling to room temperature. The liquid phase was discarded. The solid phase was ground and recrystallized from acetonitrile to yield **8b** as off-white moisture-sensitive flakes (17.2 g, 85%). ^{1}H NMR ($CDCl_3$) δ ppm: 2.82 (3H, s, CH_3), 3.06 (3H, s, S–CH_3), 4.05 (3H, s, N^+–CH_3), 7.01 (2H, d, J = 7.9, 2ArH), 7.52 (4H, m, 4ArH), 7.67 (1H, d, J = 8.1, ArH), 7.77 (1H, d, J = 7.7, ArH). ^{13}C NMR ($CDCl_3$) δ ppm: 15.0 (S–CH_3), 21.3 (CH_3), 33.5 (N^+–CH_3), 112.1, 113.5 (2× ArC), 125.9 (2ArC), 127.4, 127.8 (2× ArC), 128.4 (2ArC), 131.5, 129.5, 142.3, 149.3, 171.1 (5× ArC_q).

4.2.15. 4-Methyl-2-methylthio-thiazolopyridinium tosylate (8c). To 510 mg (2.79 mmol) 2-methylthio-thiazolopyridine (**7c**) was added 520 mg (2.79 mmol) methyl *p*-toluenesulfonate and heated to 130 °C for 1 h. After cooling the mixture to 100 °C, cyclohexane was added. Reflux was maintained for another 30 min before cooling to room temperature. The resulting precipitate was collected by filtration to yield **8c** as an off-white solid (925 mg, 90 %). ^{1}H NMR ($CDCl_3$) δ ppm: 2.27 (3H, s, CH_3), 2.80 (3H, s, S–CH_3), 4.51 (3H, s, N^+–CH_3), 7.03 (2H, d, J = 7.8, 2ArH), 7.63 (2H, d, J = 7.9, 2ArH), 7.74 (1H, m, ArH), 9.07 (1H, d, J = 7.2, ArH), 9.16 (1H, d, J = 4.3, ArH). ^{13}C NMR ($CDCl_3$) δ ppm: 16.7 (S–CH_3), 21.2 (CH_3), 44.2 (N^+–CH_3), 120.5 (ArC), 125.8 (2ArC), 128.5 (2ArC), 133.8, 139.4 (ArC_q), 139.8 (ArC), 143.0 (ArC_q), 143.5 (ArC), 154.6, 184.8 (ArC_q).

4.2.16. 4-Methyl-2-(methylthio)-oxazolopyridinium tosylate (8d). Following the procedure of **8a** were 1.97 g (11.8 mmol) 2-(methylthio)-oxazolopyridine (**7d**) and 2.20 g (11.8 mmol, 1.76 mL) methyl *p*-toluenesulfonate allowed to react to yield **8d** as a white solid (2.52 g, 61%). ^{1}H NMR ($CDCl_3$) δ ppm: 2.24 (3H, s, CH_3), 2.78 (3H, s, S–CH_3), 4.42 (3H, s, N^+–CH_3), 6.99 (2H, d, J = 8.0, 2ArH), 7.54 (2H, d, J = 8.1, 2ArH), 7.78 (1H, dd, J_1 = 8.3, J_2 = 6.4, ArH), 8.44 (1H, d, J = 8.2, ArH), 9.04 (1H, d, J = 6.4, ArH). ^{13}C NMR ($CDCl_3$) δ ppm: 15.1 (S–CH_3), 21.1 (CH_3), 42.0 (N^+–CH_3), 121.2, 125.6, 128.3 (3× ArC), 138.9 (ArC_q), 141.0 (ArC), 143.5, 147.3, 150.2, 177.8 (4× ArC_q).

4.2.17. 1-(Carboxymethyl)-4-methyl-chinolinium bromide (9). A solution of 11.5 g (80.0 mmol) lepidine and 13.9 g (100 mmol) bromoacetic acid in 40 mL EtOAc was stirred for 3 days at room temperature. The resulting precipitate was collected by filtration and dried under reduced pressure to yield **9** as an off-white solid (11.9 g, 53 %). ^{1}H NMR (CD_3OD) δ ppm: 1.99 (3H, s, CH_3), 3.79 (2H, s, CH_2), 6.95 (2H, t, J = 7.1, 2ArH), 7.13 (1H, t, J = 7.8, ArH), 7.22 (1H, d, J = 8.9, ArH), 7.48 (1H, d, J = 8.5, ArH), 8.15 (1H, d, J = 6.1, ArH). ^{13}C NMR (CD_3OD) δ ppm: 20.6 (CH_3), 58.7 (CH_2), 120.0, 123.9, 128.3 (3× ArC), 130.7 (ArC_q), 131.2, 137.0 (2× ArC), 139.6 (ArC_q), 150.6 (ArC), 162.5 (ArC_q), 168.5 (C_q).

4.2.18. General procedure for the coupling of cyanine dyes 1b and d to Fmoc-Aeg-OAll (10). To 1.00 mmol of cyanine dye **1b** or **d** in 11 mL dry DMF were added 572 mg (1.10 mmol) PyBOP, 111 mg (1.10 mmol, 120 μL) NMM, and 253 mg (1.00 mmol) PPTS under an atmosphere of argon and exclusion of light. This suspension was stirred for 2 min whereupon complete dissolution occurs. The solution is then added to a solution of 417 mg (1.00 mmol) Fmoc-Aeg-OAll hydrochloride (**10**) and 111 mg (1.10 mmol, 120 μL) NMM in 9 mL dry DMF. The resulting mixture was stirred over 16h. The volatiles were removed under reduced pressure and the residue treated with 20 mL CH_2Cl_2. The resulting precipitate was filtered off and discarded. The filtrate was purified by flash column chromatography (CH_2Cl_2/0 % MeOH → 2 % MeOH).

4.2.19. Fmoc-Aeg(YO)-OAll (12b). Yellow solid (253 mg, 33 %). TLC R_f = 0.58 (CH_2Cl_2/MeOH/HCOOH 89.5:10:0.5). ^{1}H NMR (DMSO-d_6) (two rotamers) δ ppm: 3.14 (1H, m, N–CH_2), 3.41 (2H, m, N–CH_2), 3.63 (1H, m, N–CH_2), 3.89 (3H, s, CH_3), 4.15 (1.2H, s, Gly-CH_2), 4.24 (1H, m, Fmoc-CH), 4.31 (0.8H, d, J = 6.7, Fmoc-CH_2) 4.40 (1.2H, d, J = 6.3, Fmoc-CH_2), 4.52 (0.8H, s, Gly-CH_2), 4.57 (1.2H, d, J = 5.0, All–CH_2), 4.77 (0.8H, d, J = 5.1, All–CH_2), 5.19 (0.6H, d, J_1 = 10.7, All=CH_2), 5.29 (0.6 H, d, J_1 = 17.4, All=CH_2), 5.31 (0.4H, d, J_1 = 9.5, All=CH_2), 5.43 (0.4H, d, J_1 = 17.5, All=CH_2), 5.50 (0.8H, s, CH_2), 5.70 (1.2H, s, CH_2), 5.87 (0.6H, m, All–CH), 6.04 (0.4H, m, All–CH), 6.34 (1H, s, CH), 7.31 (2H, t, J = 7.1, 2Fmoc-ArH), 7.41 (3H, m, 2Fmoc-ArH, ArH), 7.50 (1H, t, J = 7.6 Hz, ArH), 7.66 (4H, m, 2Fmoc-ArH, 2ArH), 7.80 (2H, m, 2ArH), 7.91 (4H, m, 2Fmoc-ArH, 2ArH), 8.22 (1H, m, ArH), 8.75 (1H, d, J = 8.5, ArH). ^{13}C NMR (DMSO-d_6) (two rotamers) δ ppm: 30.5 (CH_3), 37.0, 38.0 (2× N–CH_2), 46.7 (Fmoc-CH), 47.0, 47.4 (2× N–CH_2), 47.5, 48.1 (2× Gly-CH_2), 54.4, 54.9 (2× CH_2), 64.9 (All–CH_2), 65.3, 65.5 (2× Fmoc-CH_2), 65.8 (All–CH_2), 74.6, 74.7 (2× CH), 108.7,

110.8, 110.8, 117.6 (4× ArC), 117.8, 118.5 (2× All=CH_2), 120.1 (Fmoc-ArC), 122.9 (ArC_q), 124.4 (ArC), 125.0 (Fmoc-ArC), 125.7, 125.9, 126.2 (3× ArC), 127.0, 127.6 (2× Fmoc-ArC), 131.2 (ArC_q), 132.1, 132.2 (2× All–CH), 133.9 (ArC), 138.0, 138.1 (2× ArC_q), 140.7, 140.7, 143.7, 143.8 (4× Fmoc-ArC_q), 144.1 (ArC), 146.0 (ArC_q), 150.1 (ArC), 156.1, 156.5 (2× Fmoc-C_q), 161.5, 161.5 (2× ArC), 166.2, 166.5 (2× C_q), 168.4, 169.3 (2× Gly-C_q). HR-MS: *m/z* calcd for $C_{42}H_{39}N^4O^{6+}$ 695.2864, found 695.2860 $[M]^+$.

4.2.20. Fmoc-Aeg(JO)-OAll (12d). Violet solid (326 mg, 42 %). TLC $R_f = 0.68$ (CH_2Cl_2/MeOH/HCOOH 89.5:10:0.5). ^1H NMR (DMSO-d_6) (two rotamers) δ ppm: 3.17 (1H, m, N–CH_2), 3.41 (2H, m, N–CH_2), 3.64 (1H, m, N–CH_2), 4.18 (4H, m, CH_3, Gly-CH_2), 4.24 (1H, m, Fmoc-CH), 4.32 (0.8H, d, $J = 6.8$, Fmoc-CH_2) 4.41 (1.2H, d, $J = 6.6$, Fmoc-CH_2), 4.52 (0.8H, s, Gly-CH_2), 4.57 (1.2H, d, $J = 5.3$, All–CH_2), 4.77 (0.8H, d, $J = 5.5$, All–CH_2), 5.20 (0.7H, dd, $J_1 = 1.2$, $J_2 = 10.5$, All=CH_2), 5.30 (1H, m, All=CH_2), 5.43 (1.3H, m, All=CH_2, CH_2), 5.59 (1H, s, CH_2), 5.88 (0.6H, m, All–CH), 6.03 (0.4H, m, All–CH), 6.48 (1H, m, CH), 7.30 (3H, m, 2Fmoc-ArH, ArH), 7.40 (2H, t, $J = 7.30$, 2Fmoc-AcH), 7.54 (2H, m, 2ArH), 7.771 (3H, m, 2Fmoc-ArH, ArH), 7.88 (2H, d, $J = 7.4$, 2Fmoc-ArH), 8.01 (1H, d, $J = 7.5$, ArH), 8.10 (1H, d, $J = 7.7$, ArH), 8.24 (2H, m, 2ArH), 8.45 (1H, d, $J = 8.5$, ArH). ^{13}C NMR (DMSO-d_6) (two rotamers) δ ppm: 38.0 (N–CH_2), 40.2 (CH_3), 46.7 (Fmoc-CH), 47.0, 47.4 (2× N–CH_2), 48.1, 49.1 (2× Gly-CH_2), 53.9, 54.5 (2× CH_2), 64.9 (All–CH_2), 65.3, 65.5 (2× Fmoc-CH_2), 65.8 (Al–CH_2), 81.7 (CH), 108.5, 116.2, 117.3 (3× ArC), 117.8, 118.4 (2× All=CH_2), 118.5 (ArC), 120.1 (Fmoc-ArC), 122.8 (ArC_q), 124.9 (ArC), 125.0 (Fmoc-ArC), 125.7 (ArC), 127.0, 127.6 (2× Fmoc-ArC), 132.1, 132.2 (2× All–CH), 132.4, 135.8 (2× ArC), 138.0, 138.0 (2× ArC_q), 140.7, 140.7 (2× Fmoc-ArC_q), 142.8 (ArC), 143.7, 143.8 (2× Fmoc-ArC_q), 145.1 (ArC_q), 150.0 (ArC), 153.7 (ArC_q), 156.1, 156.5 (2× Fmoc-C_q), 166.2, 166.5 (2× C_q), 168.4, 169.4 (2× Gly-C_q), 171.4 (ArC_q). HR-MS: *m/z* calcd for $C_{41}H_{38}N_5O_6^+$ 696.2817, found 696.2814 $[M]^+$.

4.2.21. Fmoc-Aeg(YO)-OH (13b). To 217 mg (0.279 mmol) Fmoc-Aeg(YO)-OAll (**12b**) in 20 mL dry and degassed THF were added 29.8 mg (0.279 mmol, 30.5 μL) *N*-methylaniline and 16.1 mg (0.014 mmol) $Pd(PPh_3)_4$ under an atmosphere of Ar and exclusion of light. This solution was stirred for 16 h. Afterward all volatiles were removed and the residue was purified by flash column chromatography (CH_2Cl_2, 0% MeOH, 0% HCOOH → 4% MeOH, 0.5% HCOOH) to yield **13b** as a red solid (198 g, 96 %). TLC $R_f = 0.20$ (CH_2Cl_2/MeOH/HCOOH 89.5:10:0.5). ^1H NMR (DMSO-d_6) (two rotamers) δ ppm: 3.16 (1H, m, N–CH_2), 3.40 (2H, m, N–CH_2), 3.61 (1H, m, N–CH_2), 3.87 (3H, m, CH_3), 4.03 (2H, s, Gly-CH_2), 4.22 (1H, m, Fmoc-CH), 4.30 (0.8H, d, $J = 6.9$, Fmoc-CH_2) 4.39 (1.2H, d, $J = 6.6$, Fmoc-CH_2), 5.47 (1H, s, CH_2), 5.68 (1H, s, CH_2), 6.30 (0.5H, s, CH), 6.32 (0.5H, s, CH), 7.31 (2H, t, $J = 7.3$, 2Fmoc-ArH), 7.40 (3H, m, 2Fmoc-ArH, ArH), 7.49 (1H, t, $J = 7.7$, ArH), 7.67 (4H, m, 2Fmoc-ArH, 2ArH), 7.78 (2H, m, 2ArH), 7.89 (4H, m, 2Fmoc-ArH, 2ArH), 8.24 (1H, m, ArH), 8.73 (1H, d, $J = 7.9$, ArH). ^{13}C NMR (DMSO-d_6) (two rotamers) δ ppm: 30.5 (CH_3), 37.9 (N–CH_2), 46.7 (Fmoc-CH), 47.0, 47.2 (2× N–CH_2), 47.9, 49.2 (2× Gly-CH_2), 54.4, 55.0 (CH_2), 65.4, 65.5 (2× Fmoc-CH_2), 74.6, 74.7 (2× CH), 108.7, 110.8, 110.8, 117.7 (4× ArC), 120.1, 120.1 (2× Fmoc-ArC),122.9, 123 (2× ArC_q), 124.5 (ArC), 125.1 (Fmoc-ArC), 125.8, 125.9, 126.2 (3× ArC), 127.0, 127.6, 127.6 (3× Fmoc-ArC), 131.3 (ArC_q), 132.9, 133.0 (2× ArC), 138.1, 138.2 (2× ArC_q), 140.7, 140.7, 143.8, 143.8 (4× Fmoc-ArC_q), 144.2, 144.5 (ArC), 146.1, 150.2 (2× ArC_q), 156.1, 156.5 (2× Fmoc-C_q), 161.6, 161.6 (2× ArC_q), 166.0, 166.4 (2× C_q), 170.2, 171.1 (2× Gly-C_q). HR-MS: *m/z* calcd for $C_{39}H_{35}N_4O_6^+$ 655.2551, found 695.2543 $[M]^+$.

4.2.22. Fmoc-Aeg(JO)-OH (13d). Following the procedure of **12b** were 395 mg (0.509 mmol) Fmoc-Aeg(JO)-OAll (**12d**), 54.5 mg (0.509 mmol, 55.7 μL) *N*-methylaniline, and 29.4 mg (0.025 mmol) $Pd(PPh_3)_4$ in 40 mL dry and degassed THF allowed to react o yield **13d** as a violet solid (291 mg, 78 %). TLC $R_f = 0.23$ (CH_2Cl_2/MeOH/HCOOH 89.5:10:0.5). ^1H NMR (DMSO-d_6) (two rotamers) δ ppm: 3.15 (1H, m, N–CH_2), 3.39 (2H, m, N–CH_2), 3.59 (1H, m, N–CH_2), 4.03 (1H, m, Gly-CH_2), 4.20 (3H, s, CH_3), 4.25 (1H, m, Fmoc-CH), 4.30 (0.7H, d, $J = 6.8$, Fmoc-CH_2) 4.39 (1.3H, d, $J = 6.9$, Fmoc-CH_2), 5.36 (1H, s, CH_2), 5.57 (1H, s, CH_2), 6.54 (1H, m, CH), 7.32 (3H, m, 2Fmoc-ArH, ArH), 7.41 (2H, t, $J = 7.8$, 2Fmoc-ArH), 7.56 (2H, m, 2ArH), 7.68 (3H, m, 2Fmoc-ArH, ArH), 7.88 (2H, d, $J = 7.5$, 2Fmoc-ArH), 8.05 (1H, d, $J = 6.1$, ArH), 8.15 (1H, d, $J = 7.7$, ArH), 8.29 (2H, m, 2ArH), 8.50 (1H, d, $J = 8.5$, ArH). ^{13}C NMR (DMSO-d_6) (two rotamers) δ ppm: 37.9 (N–CH_2), 40.2 (CH_3), 46.6 (Fmoc-CH), 47.0, 47.2 (2× N–CH_2), 47.8, 49.1 (2× Gly-CH_2), 54.0, 54.5 (2× CH_2), 65.3, 65.5 (2× Fmoc-CH_2), 81.5 (CH), 108.5, 116.2, 117.4, 118.4 (4× ArC), 120.1 (Fmoc-ArC), 122.9 (ArC_q), 125.0 (ArC), 125.0 (Fmoc-ArC), 125.7 (ArC), 127.0, 127.5, 127.6 (3× Fmoc-ArC), 132.4, 135.8 (2ArC), 138.0, 138.1 (2× ArC_q), 140.6, 140.7 (2× Fmoc-ArC_q), 142.9, 143.1 (2× ArC), 143.7, 143.8 (2× Fmoc-ArC_q), 145.2, 148.6, 150.1, 154.0 (4× ArC_q), 156.1, 156.5 (2× Fmoc-C_q), 166.2, 166.6 (2× C_q), 170.1, 171.1 (Gly-C_q), 171.5 (ArC_q). HR-MS: m/z calcd. for $C_{38}H_{34}N_5O_6^+$ 656.2504, found 656.2497 $[M]^+$.

4.3. Solid-phase synthesis of labeled PNA conjugates

4.3.1. Loading of Novagen TGR resin. The resin (250 mg, 0.29 μmol/g) was allowed to swell in 10 mL DMF for 30 min. For Fmoc removal the resin was twice treated with 1.5 mL of DMF/piperidine (4:1, v/v) and subsequently washed with DMF (5× 2 mL), CH_2Cl_2 (5× 2 mL), and DMF (5× 2 mL). For preactivation PyBOP (130.1 mg, 250 μmol) and NMM (37.9 mg, 375 μmol) were added to a solution of Fmoc-protected glycine (74.3 mg, 250 μmol) in DMF (1.5 mL). After 3 min, the mixture was added to the resin. After 2.5 h, the resin was washed with DMF (5× 2 mL), CH_2Cl_2 (5× 2 mL), and DMF (5× 2 mL). For capping, the resin was treated twice with 1.5 mL of a solution of Ac_2O/pyridine (1:9, v/v). The resin was washed with DMF (5× 2 mL) and CH_2Cl_2 (5× 2 mL), and finally dried under reduced pressure.

4.3.2. Linear solid-phase synthesis. Fmoc-glycine loaded resin (ca. 2 μmol) was allowed to swell in DMF (2 mL). After 30 min, the resin was transferred to a synthesizer reactor. The resin was washed (2× 200 μL DMF).

4.3.3. Fmoc cleavage. A solution of DMF/piperidine (4:1, v/v, 200 μL) was added to the resin. After 2 min, the procedure was repeated. Finally the resin was washed with DMF (7× 200 μL).

4.3.4. Coupling of Fmoc-Bhoc-PNA-monomers. A preactivation vessel was charged with a 0.6 M HCTU solution in NMP (13.3 μL), a 4 M NMM solution in DMF (4 μL), and a 0.2 M PNA monomer solution in NMP (40 μL). After 2 min, 40 μL of preactivation solution was transferred to the resin. After 30 min, the resin was washed with DMF (2× 200 μL).

4.3.5. Coupling of Fmoc-Aeg(dye)-OH (12a,b,d) and Fmoc-Aeg(Alloc)-OH (11). A preactivation vessel was charged with a 0.6 M HCTU solution in NMP (13.3 μL), a 4 M NMM solution in DMF (4 μL), and a 0.2 M solution of **13a**, **b**, **d** or **11**, and PPTS in NMP (40 μL). After 2 min, 40 μL of the preactivation solution was transferred to the resin. After 60 min, the procedure was repeated and finally the resin was washed with DMF (2× 200 μL).

4.3.6. Capping. One hundred microliters of a solution of Ac_2O/2,6-lutidine/DMF (5:6:89, v/v/v) was added to the resin. After 2 min, the resin was washed with DMF (2× 200 μL).

4.3.7. Cleavage. A solution of cysteine methyl ester hydrochloride (7.5 mg, 45 μmol) in 1.5 mL of a solution of TFA/*m*-cresol/H_2O (93:5:2, v/v/v) was passed through the dried resin in 30 min. The resin was washed with TFA (1× 200 μL). The combined filtrates were concentrated in vacuo.

4.3.8. Purification. To the concentrated cleavage solution was added cold diethyl ether. The precipitate was collected by centrifugation and disposal of the supernatant. The residue was dissolved in water and precleaned by using a water-equilibrated Sep-pak® C18 cartridge. Colored eluates obtained upon elution with MeCN/H_2O (70:30, v/v) were analyzed by HPLC and MALDI-TOF/MS, and purified by semipreparative HPLC. Determination of yields: Purified PNA was dissolved in 250 μL of water. An aliquot of 5 μL was diluted to 1 mL and the optical density was measured at 260 nm by using a quartz cuvette with a 10 mm path length. The sample concentration was calculated by using oligo calculation at www.gensetoligos.com and ε(TO) = 6600 $Lmol^{-1}$, ε(YO) = 7100 $Lmol^{-1}$, ε(MO) = 4500 $Lmol^{-1}$, and ε(JO) = 7200 $Lmol^{-1}$.

4.3.9. $^{Ac\text{-}N}$gccgta Aeg(TO) atagccgGly^{CONH_2} (19a). Thirteen milligrams (ca. 4 μmol) of Fmoc-glycine loaded Novasyn TGR Rink-Amide resin was used. OD_{260} = 20.58 (151 nmol, 3.7%); t_R = 12.31 min; MALDI-TOF-MS (sinapinic acid): *m/z* calcd for $C_{167}H_{202}N_{83}O_{42}S^+$: 4073, found 4073 $[M(average)]^+$.

4.3.10. $^{Ac\text{-}N}$gccgta Aeg(YO) atagccgGly^{CONH_2} (19b). 11.1 mg (ca. 2 μmol) of Fmoc-glycine loaded Novasyn TGR Rink-Amide resin was used. OD_{260} = 19.1 (144 nmol, 7.2%); t_R = 10.79 min; MALDI-TOF-MS (sinapinic acid): *m/z* calcd for $C_{167}H_{202}N_{83}O_{43}^+$ 4060, found 4061 $[M(average)]^+$.

4.3.11. $^{Ac\text{-}N}$gccgta Aeg(MO) atagccgGly^{CONH_2} (19c). 1.1 mg (ca. 2 μmol) of Fmoc-glycine loaded Novasyn TGR Rink-Amide resin was used to first assemble resin (**15c**), which was washed with dry and degassed CH_2Cl_2 (5× 1 mL) and treated with a solution of 12 μmol Me_2N-H·BH_3 and 2 μmol $Pd(PPh_3)_4$ in 1 mL of dry and degassed CH_2Cl_2 for 10 min for Alloc removal. The resin was washed consecutively with dry and degassed CH_2Cl_2, dry DMF, and dry and degassed CH_2Cl_2 (each 3× 1 mL), and the treatment with Me_2NH·BH_3 and $Pd(PPh_3)_4$ is repeated for 20 min. Finally the resin was washed consecutively with CH_2Cl_2 (2× 1 mL), DMF (3× 1 mL), dioxane/H_2O (2× 1 mL), MeOH (1× 1 mL), DMF (3× 3 mL), and CH_2Cl_2 (3× 3 mL). For labeling with MO-dye 10 μmol of **1c** was dissolved in dry DMF (0.1 M) and 9.8 μmol PyBOP, 10 μmol PPTS, and 12 μmol NMM were added and the resulting mixture was shaken for 5 min at room temperature. With this solution the resin was treated for 2 h. The procedure was repeated three times. Finally the resin was washed with DMF (5× 1 mL) and CH_2Cl_2 (10× 1 mL). Liberation from the resin and deprotection was carried out as described in the general methods. OD_{260} = 4.68 (35.3 nmol, 1.8 %); t_R = 11.11 min; MALDI-TOF-MS (sinapinic acid): *m/z* calcd For $C_{166}H_{201}N_{84}O_{42}S_1^+$ 4077, found 4079 $[M(average)]^+$.

4.3.12. $^{Ac\text{-}N}$gccgta Aeg(JO) atagccgGly^{CONH_2} (19d). 11.1 mg (ca. 2 μmol) of Fmoc-glycine loaded Novasyn TGR Rink-Amide resin was used. OD_{260} = 19.0 (145 nmol, 7.2 %); t_R = 10.37 min; M ALDI-TOF-MS (sinapinic acid): *m/z* calcd For $C_{166}H_{201}N_{84}O_{43}^+$ 4061, found 4062 $[M(average)]^+$.

References and notes

1. Seitz, O.; Bergmann, F.; Heindl, D. *Angew. Chem. Int. Ed.* **1999**, *38*, 2203–2206.
2. Jarikote, D. V.; Köhler, O.; Socher, E.; Seitz, O. *Eur. J. Org. Chem.* **2005**, 3187–3195.
3. Köhler, O.; Jarikote, D. V.; Seitz, O. *Chem. Commun.* **2004**, 2674–2675.
4. Köhler, O.; Seitz, O. *Chem. Commun.* **2003**, 2938–2939.
5. Köhler, O.; Venkatrao, D.; Jarikote, D. V.; Seitz, O. *ChemBioChem* **2005**, *6*, 69–77.
6. Saito, Y.; Hanawa, K.; Motegi, K.; Omoto, K.; Okamoto, A.; Saito, I. *Tetrahedron Lett.* **2005**, *46*, 7605–7608.
7. Okamoto, A.; Tanaka, K.; Fukuta, T.; Saito, I. *ChemBioChem* **2004**, *5*, 958–963.
8. Okamoto, A.; Tanaka, K.; Fukuta, T.; Saito, I. *J. Am. Chem. Soc.* **2003**, *125*, 9296–9297.
9. Okamoto, A.; Kanatani, K.; Saito, I. *J. Am. Chem. Soc.* **2004**, *126*, 4820–4827.

10. Okamoto, A.; Tainaka, K.; Saito, I. *J. Am. Chem. Soc.* **2003**, *125*, 4972–4973.
11. Géci, I.; Filichev, V. V.; Pedersen, E. B. *Bioconjugate Chem.* **2006**, *17*, 950–957.
12. Yamana, K.; Iwase, R.; Furutani, S.; Tsuchida, H.; Zako, H.; Yamaoka, T.; Murakami, A. *Nucleic Acids Res.* **1999**, *27*, 2387–2392.
13. Christensen, U. B.; Pedersen, E. B. *Helv. Chim. Acta* **2003**, *86*, 2090–2097.
14. Mayer-Enthart, E.; Wagenknecht, H. A. *Angew. Chem. Int. Ed.* **2006**, *45*, 3372–3375.
15. Yamana, K.; Zako, H.; Asazuma, K.; Iwase, R.; Nakano, H.; Murakami, A. *Angew. Chem. Int. Ed.* **2001**, *40*, 1104–1106.
16. Ergen, E.; Weber, M.; Jacob, J.; Herrmann, A.; Müllen, K. *Chem. Eur. J.* **2006**, *12*, 3707–3713.
17. Dobson, N.; McDowell, D. G.; French, D. J.; Brown, L. J.; Mellor, J. M.; Brown, T. *Chem. Commun.* **2003**, 1234–1235.
18. French, D. J.; Archard, C. L.; Andersen, M. T.; McDowell, D. G. *Mol. Cell. Probes* **2002**, *16*, 319–326.
19. French, D. J.; Archard, C. L.; Brown, T.; McDowell, D. G. *Mol. Cell. Probes* **2001**, *15*, 363–374.
20. McDowell, D.; French, D.; Brown, T. *J. Med. Genet.* **2000**, *37*, S17.
21. Yamane, A. *Nucleic Acids Res.* **2002**, *30*, e97.
22. Kodama, S.; Asano, S.; Moriguchi, T.; Sawai, H.; Shinozuka, K. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2685–2688.
23. Dose, C.; Ficht, S.; Seitz, O. *Angew. Chem Int. Ed.* **2006**, *45*, 5369–5373.
24. Abe, H.; Kool, E. T. *J. Am. Chem. Soc.* **2004**, *126*, 13980–13986.
25. Huber, R.; Amann, N.; Wagenknecht, H. A. *J. Org. Chem.* **2004**, *69*, 744–751.
26. Valis, L.; Amann, N.; Wagenknecht, H. A. *Org. Biomol. Chem.* **2005**, *3*, 36–38.
27. Asseline, U.; Chassignol, M.; Aubert, Y.; Roig, V. *Org. Biomol. Chem.* **2006**, *4*, 1949–1957.
28. Privat, E.; Melvin, T.; Asseline, U.; Vigny, P. *Photochem. Photobiol.* **2001**, *74*, 532–541.
29. Svanvik, N.; Westman, G.; Wang, D. Y.; Kubista, M. *Anal. Biochem.* **2000**, *281*, 26–35.
30. Svanvik, N.; Nygren, J.; Westman, G.; Kubista, M. *J. Am. Chem. Soc.* **2001**, *123*, 803–809.
31. Deligeorgiev, T. G.; Gadjev, N. I.; Timtcheva, II; Maximova, V. A.; Katerinopoulos, H. E.; Foukaraki, E. *Dyes Pigment.* **2000**, *44*, 131–136.
32. Vasilev, A.; Deligeorgiev, T.; Gadjev, N.; Drexhage, K. H. *Dyes Pigment.* **2005**, *66*, 135–142.
33. Haugland, R. P.; Yue, S. T. WO 00/66664, 2000.
34. Smith, K. *Sulfur Lett.* **1994**, *18*, 79–95.
35. Walczynski, K.; Zuiderveld, O. P.; Timmerman, H. *Eur. J. Med. Chem.* **2005**, *40*, 15–23.
36. Chu-Moyer, M. Y.; Berger, R. *J. Org. Chem.* **1995**, *60*, 5721–5725.
37. Ramadas, K.; Srinivasan, N. *Synth. Commun.* **1995**, *25*, 227–234.
38. Rye, H. S.; Yue, S.; Wemmer, D. E.; Quesada, M. A.; Haugland, R. P.; Mathies, R. A.; Glazer, A. N. *Nucleic Acids Res.* **1992**, *20*, 2803–2812.
39. Zhou, X. F.; Peng, Z. H.; Geise, H. J.; Peng, B. X.; Li, Z. X.; Yan, M.; Dommisse, R.; Carleer, R.; Claeys, M. *J. Imaging Sci. Technol.* **1995**, *39*, 244–252.
40. Brooker, L. G. S.; Keyes, G. H.; Williams, W. W. *J. Am. Chem. Soc.* **1942**, *64*, 199–210.
41. Seitz, O.; Köhler, O. *Chem. Eur. J.* **2001**, *7*, 3911–3925.
42. Ficht, S.; Röglin, L.; Ziehe, M.; Breyer, D.; Seitz, O. *Synlett* **2004**, *14*, 2525–2528.
43. Petty, J. T.; Bordelon, J. A.; Robertson, M. E. *J. Phys. Chem. B* **2000**, *104*, 7221–7227.