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Efficient functional molecule incorporation method to functionalized peptide nucleic acid (PNA): use in synthesis of labeled PNA oligomers

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Abstract—A novel efficient synthetic method for a functionalized PNA (peptide nucleic acid) is described, in which a functional molecule is incorporated in place of a nucleobase. Novel ω-AA–^{Boc}PNA–OH (**20**–**24**, AA=amino acid) were designed as PNA precursor monomer units into which functional molecules could be incorporated efficiently. Compounds **20**–**24** reacted quantitatively with OSu (*N*-hydroxysuccinimidyl) active ester derivatives and isothiocyanate derivatives of commercial functional molecules to give target functionalized PNA monomer units **25**–**53**. Various types of functionalized PNA monomer units could be efficiently incorporated into multiple predetermined positions in a PNA oligomer by SPPS (solid phase peptide synthesis) in the same way as for the four A(Cbz), G(Cbz), C(Cbz), and T PNA monomer units.
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1. Introduction

Peptide nucleic acid (PNA) is a modified nucleic acid in which the sugar–phosphate backbone of a nucleic acid has been converted into an *N*-(2-aminoethyl)glycine backbone.[1] The sugar–phosphate backbones of DNA/RNA are negatively charged under physiological conditions and exhibit electrostatic repulsion between complementary strands. However, since the backbone structure of PNA itself has no charge, there is no electrostatic repulsion between PNA and the complementary DNA. PNA, therefore, has a high duplex stability and a high sequence recognition ability in comparison with conventional nucleic acids.[2] Furthermore, since PNA is significantly stable against cellular nucleases/proteases and is not decomposed, thereby its application in gene therapy as an antisense molecule has been investigated.[3] Modifying conventional techniques that employ DNA as a medium so that they can be used with PNA can compensate for the defects of DNA that could not be overcome previously. For example, it is possible to apply PNA to the 'DNA microarray technology' that carries out a systematic analysis of a large amount of genetic information at high-throughput,[4] and to the 'molecular beacon'[5a–c] and 'reporter probe'[5d–f] that has been developed recently as a probe that can detect a full-matched sequence specifically by fluorescence. Since these techniques use DNA as a medium, which has poor enzyme resistance, when employing these techniques it is necessary to carry out precise sampling. Satisfying this requirement is the key to enhance the above-mentioned techniques. Also, since PNA is completely resistant to enzymes, the use of PNA as a replacement for DNA in the DNA microarray technology, the molecular beacon, and the reporter probe is anticipated to eliminate the defects of the above-mentioned techniques and to derive further advantages. There are a large number of fields, in addition to the DNA microarray technology, the molecular beacon, and the reporter probe, that are anticipated to advance as a result of the use of PNA, and in these fields it is necessary to efficiently functionalize PNA, that is to say, to design a novel functionalized PNA monomer by the efficient introduction of a functional molecule into a PNA monomer in place of a nucleobase. Because of the fact that the functionalized PNA monomer in the PNA oligomer takes three-dimensional positions into consideration, it is more effective than the PNA oligomer conjugated with only the functionalized molecule in the point that complementary strand can be strictly recognized and detected (Chart 1).

With regard to a general conventional method for the synthesis of PNA monomer units, a carboxylic acid derivative **3** in which a linker has been added to a functional molecule **1** is condensed with ethyl *N*-(2-Boc-aminoethyl)glycinate (^{Boc}PNA–OEt, **4**), and the product is then subjected to alkaline hydrolysis to give the target product **8** (path A, Scheme 1).[6] However, since the functional molecule **1**, in particular,

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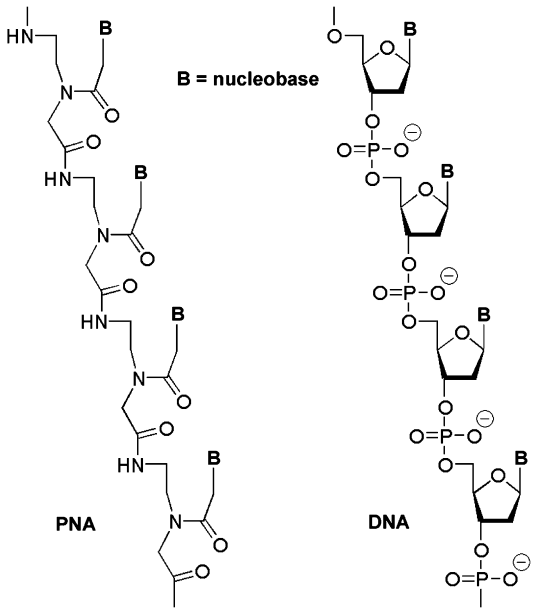


Chart 1.

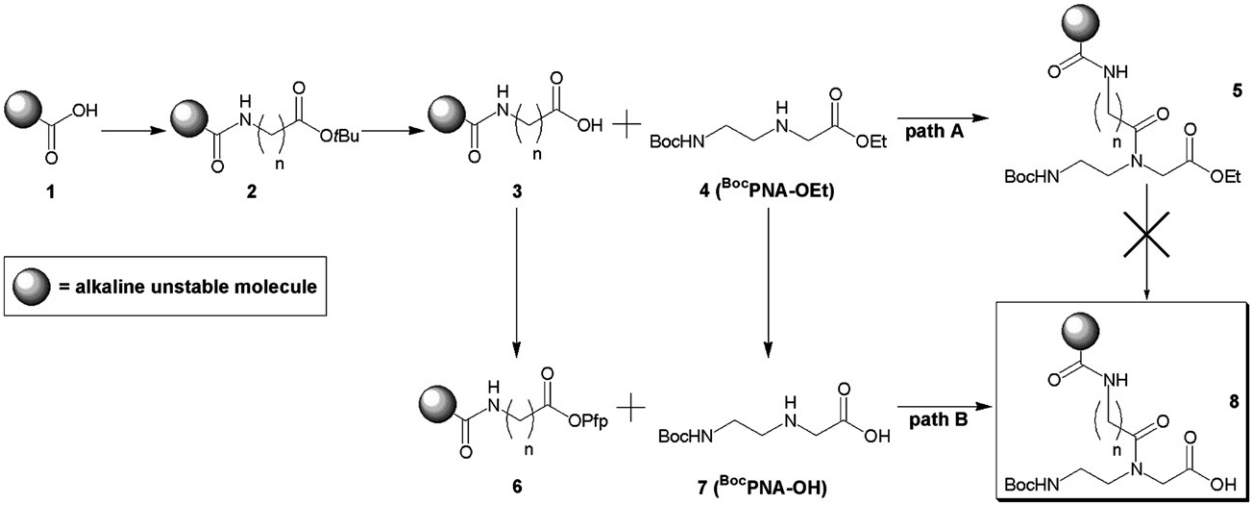
photoactive molecules having a chromophore, are unstable under alkaline conditions and susceptible to decomposition,[7] success in the synthesis of functionalized PNA monomer unit **8** via **5** has so far been limited. Recently, we have succeeded in quantitatively synthesizing functionalized PNA monomer unit **8** under mild condensation conditions using the pentafluorophenyl active ester derivative **6** of **3** and the key compound *N*-(2-Boc-aminoethyl)glycine (^{Boc}PNA–OH; **7**) (path B, Scheme 1).[8] At the same time, we have succeeded in incorporating a functional molecule into a desired position of a PNA oligomer using **8**.

However, when incorporating **1**, it is necessary to carry out (1) incorporation of an ω-amino acid derivative corresponding to the linker (synthesis of **2** and **3**), (2) conversion into the pentafluorophenyl active ester derivative **6**, and then (3) condensation with **7**. This method cannot be said to be efficient in terms of the number of steps when forming monomer units using various types of functional molecules **1**. Furthermore, the only linker used is Gly–OH, but in order to optimize the functions of the functional molecules it is necessary to prepare linkers having different lengths. Moreover, since many functional molecules, in particular, fluorescent label compounds, are very expensive, it is essential to establish a quantitative synthetic method that is efficient and has fewer steps. The number of steps can be greatly reduced by condensing the active ester derivative of a functional molecule with a PNA precursor into which a linker section has already been incorporated, rather than by forming a PNA monomer unit after incorporating the linker into the functional molecule. Furthermore, many of the expensive functional molecules are commercially available as active ester derivatives such as FAM–OSu (FAM=5(6)-carboxyfluorescein) and TAMRA–OSu (TAMRA=5(6)-carboxy-tetramethylrhodamine) and, in practice, by simply condensing them with PNA precursors, functionalized PNA monomer units having various types of linkers can be synthesized in one step. We report here a method for efficiently synthesizing functionalized PNA monomer units in which a PNA nucleobase is replaced with a functional molecule using ω-AA–^{Boc}PNA–OH as the PNA precursor. Furthermore, when functionalized PNA oligomers are synthesized using the functionalized PNA monomer units designed above, multiple different functional molecules can be incorporated into desired positions of the oligomers, which are also be reported below.

2. Results and discussion

2.1. Synthesis of PNA precursor monomer units (ω-AA–^{Boc}PNA–OH, 20–24)

Synthesis of ω-AA–^{Boc}PNA–OH (**20**–**24**) was carried out following the standard method of Dueholm et al.[6] That is, commercial Cbz–ω-AA–OH (*n*=1–5) (Cbz=benzyloxycarbonyl) and ^{Boc}PNA–OEt (**4**) were condensed to give Cbz–ω-AA–^{Boc}PNA–OEt (**9**–**13**). This was then subjected to alkaline hydrolysis to convert it into Cbz–ω-AA–^{Boc}PNA–OH (**14**–**18**) and finally to catalytic hydrogenolysis to give



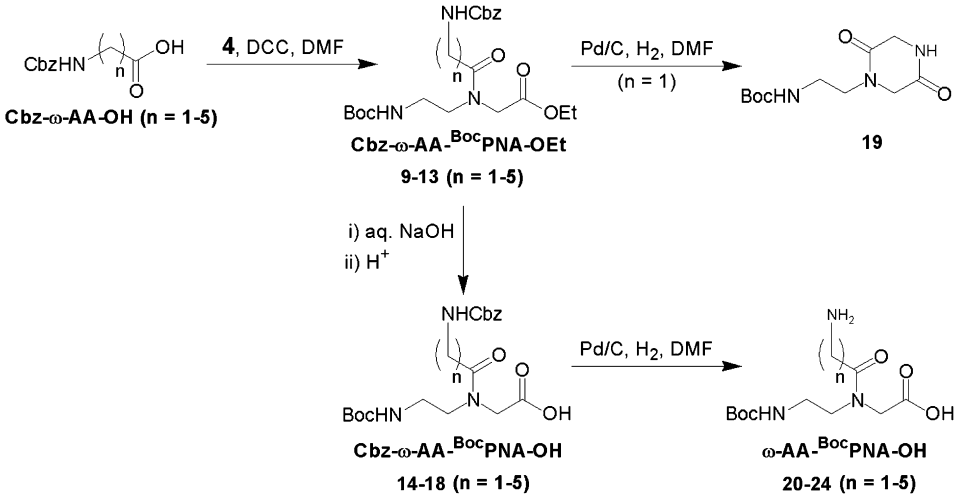
Scheme 1. Synthetic strategy for functionalized PNA monomer **8**.

the target **20**–**24**. Catalytic hydrogenolysis of Cbz–ω-Gly–^{Boc}PNA–OEt (**9**) was attempted before the alkaline hydrolysis, but the target ω-AA–^{Boc}PNA–OEt could not be obtained and, instead, the cyclic compound **19** was formed. It is surmised that this was generated by nucleophilic attack on the ethyl ester by an amino group formed by the catalytic hydrogenolysis. Since a six-membered ring structure as shown in **19** was formed when glycine was used as the linker, if a longer linker was used a cyclic compound as shown in **19** might not be formed. However, since the cyclization above did not occur when **9**–**13** was subjected to alkaline hydrolysis beforehand, it was decided to employ the first method (Scheme 2).

Synthesis of **20**–**24** proceeded efficiently in all the steps (Table 1). During this, it was found that the length of the linker did not affect the synthesis. Furthermore, since the starting materials used in this synthesis were comparatively inexpensive, this synthetic method and the PNA precursor monomer units had a very good cost-performance ratio.

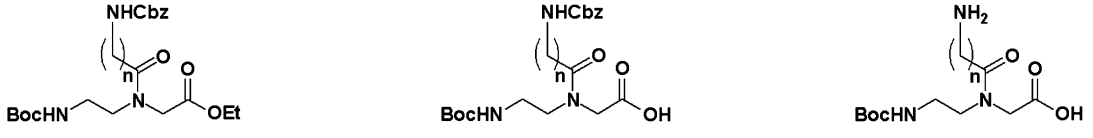
2.2. Synthesis of functionalized PNA monomer units (25–53) using ω-AA–^{Boc}PNA–OH

Since synthesis of the PNA precursor monomer units **20**–**24** was successful, next investigated was whether or not a functional molecule could be incorporated into **20**–**24** efficiently. Since **20**–**24** have both a free carboxylic group and a free amino group, they cannot be directly condensed with a functional molecule containing a free carboxylic acid derivative. It is therefore necessary to convert the functional molecule into a derivative having some type of condensing ability such as an active ester derivative. In general, an OSu derivative or an OPfp (pentafluorophenol) derivative is used as the active ester derivative, and an isothiocyanate derivative is also used. Among them, many of the fluorescent compounds represented by TAMRA and FAM are very expensive, but fortunately since most of them are commercially available as their OSu derivatives, their use has the advantage that functionalized PNA monomer units can be formed in one step (Scheme 3). Therefore, we investigated the possibility

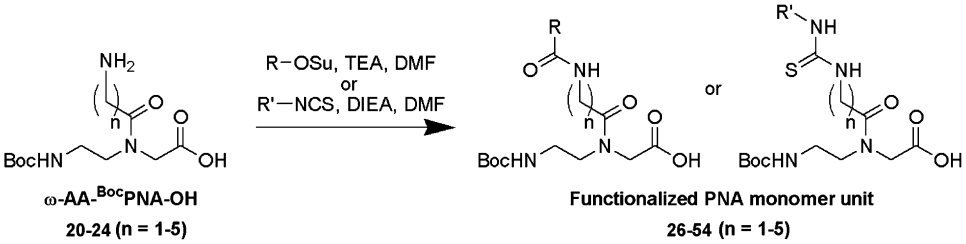


Scheme 2. Preparation of PNA precursor monomer units ω-AA–^{Boc}PNA–OH (**20**–**24**).

Table 1

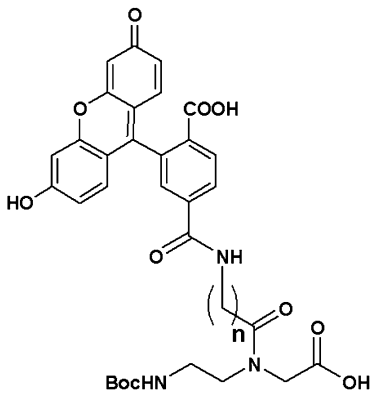


n=1	Cbz–Gly–^{Boc}PNA–OEt **9** (86% from **8**)	Cbz–Gly–^{Boc}PNA–OH **14** (82% from **9**)	Gly–^{Boc}PNA–OH **20** (89% from **14**)
n=2	Cbz–β-Ala–^{Boc}PNA–OEt **10** (86% from **8**)	Cbz–β-Ala–^{Boc}PNA–OH **15** (82% from **10**)	β-Ala–^{Boc}PNA–OH **21** (90% from **15**)
n=3	Cbz–GABA–^{Boc}PNA–OEt **11** (84% from **8**)	Cbz–GABA–^{Boc}PNA–OH **16** (88% from **11**)	GABA–^{Boc}PNA–OH **22** (83% from **16**)
n=4	Cbz–C4–^{Boc}PNA–OEt **12** (88% from **8**)	Cbz–C4–^{Boc}PNA–OH **17** (86% from **12**)	C4–^{Boc}PNA–OH **23** (67% from **17**)
n=5	Cbz–C5–^{Boc}PNA–OEt **13** (79% from **8**)	Cbz–C5–^{Boc}PNA–OH **18** (87% from **13**)	C5–^{Boc}PNA–OH **24** (79% from **18**)

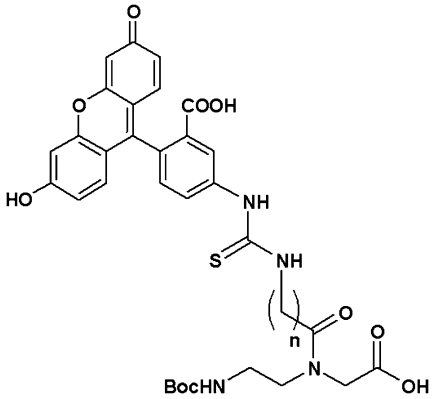


Scheme 3. Preparation of functionalized PNA monomers **25**–**53**.

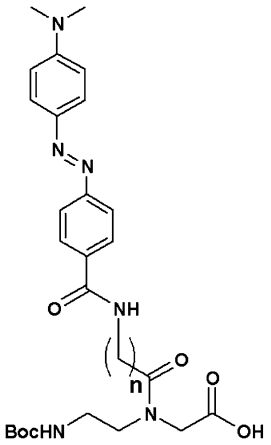
Table 2



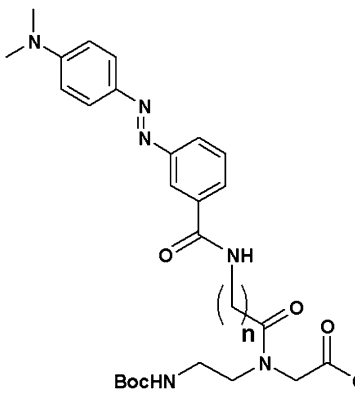
FAM–ω-AA–^{Boc}PNA–OH



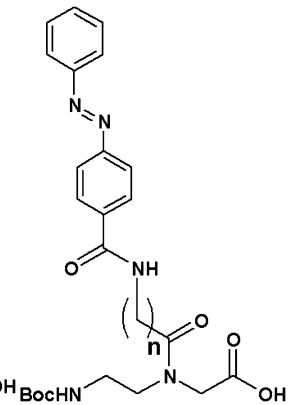
Fluorescein–ω-AA–^{Boc}PNA–OH



Dabcyl–ω-AA–^{Boc}PNA–OH

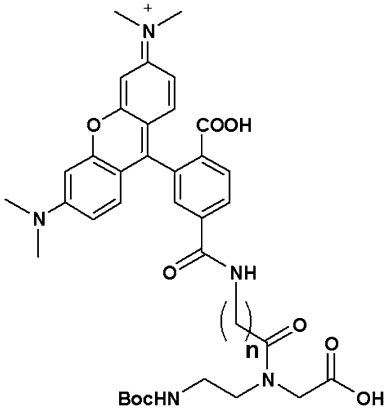


m-MR–ω-AA–^{Boc}PNA–OH

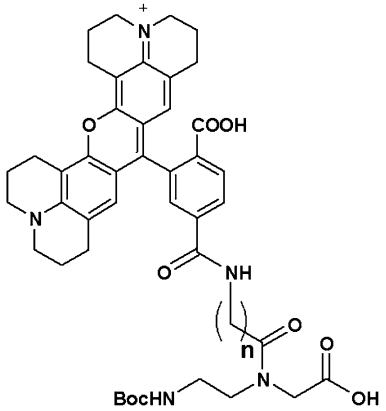


Azo–ω-AA–^{Boc}PNA–OH

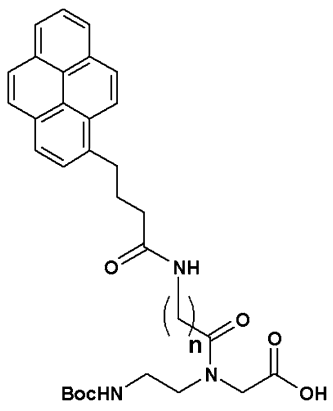
n=1 FAM–Gly–^{Boc}PNA–OH **25** (quant. from **20**)	Fluorescein–Gly–^{Boc}PNA–OH **30** (quant. from **20**)	Dabcyl–Gly–^{Boc}PNA–OH **35** (91% from **20**)	*m*-MR–Gly–^{Boc}PNA–OH **40** (quant. from **20**)	Azo–Gly–^{Boc}PNA–OH **45** (quant. from **20**)
n=2 FAM–β-Ala–^{Boc}PNA–OH **26** (quant. from **21**)	Fluorescein–β-Ala–^{Boc}PNA–OH **31** (83% from **21**)	Dabcyl–β-Ala–^{Boc}PNA–OH **36** (89% from **21**)	*m*-MR–β-Ala–^{Boc}PNA–OH **41** (89% from **21**)	Azo–β-Ala–^{Boc}PNA–OH **46** (82% from **21**)
n=3 FAM–GABA–^{Boc}PNA–OH **27** (quant. from **22**)	Fluorescein–GABA–^{Boc}PNA–OH **32** (quant. from **22**)	Dabcyl–GABA–^{Boc}PNA–OH **37** (85% from **22**)	*m*-MR–GABA–^{Boc}PNA–OH **42** (91% from **22**)	Azo–GABA–^{Boc}PNA–OH **47** (88% from **22**)
n=4 FAM–C4–^{Boc}PNA–OH **28** (quant. from **23**)	Fluorescein–C4–^{Boc}PNA–OH **33** (94% from **23**)	Dabcyl–C4–^{Boc}PNA–OH **38** (98% from **23**)	*m*-MR–C4–^{Boc}PNA–OH **43** (91% from **23**)	Azo–C4–^{Boc}PNA–OH **48** (92% from **23**)
n=5 FAM–C5–^{Boc}PNA–OH **29** (quant. from **24**)	Fluorescein–C5–^{Boc}PNA–OH **34** (quant. from **24**)	Dabcyl–C5–^{Boc}PNA–OH **39** (98% from **24**)	*m*-MR–C5–^{Boc}PNA–OH **44** (95% from **24**)	Azo–C5–^{Boc}PNA–OH **49** (87% from **24**)



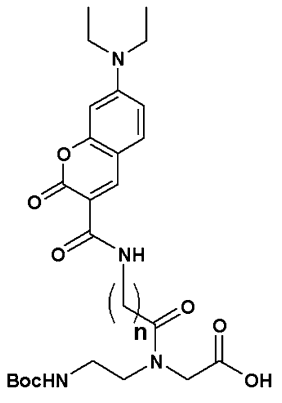
TAMRA–Gly–^{Boc}PNA–OH **50** (quant. from **20**)



ROX–Gly–^{Boc}PNA–OH **51** (quant. from **20**)



Pyrene–Gly–^{Boc}PNA–OH **52** (quant. from **20**)



Coumarine–Gly–^{Boc}PNA–OH **53** (quant. from **20**)

of efficiently incorporating a series of OSu derivatives into **20**–**24**. At the same time, a similar investigation was carried out using inexpensive isothiocyanate derivatives such as FITC (fluorescein isothiocyanate).

When **20**–**24** were reacted with various functional molecules, it was found that the condensation reaction proceeded substantially and quantitatively, and the target functionalized PNA monomer units **25**–**53** could be synthesized (Table 2).

In this stage, the length of the linker did not affect the reactivity. This result suggests that, even when very expensive functional molecules are used in the reaction, since functionalized PNA monomer units are obtained quantitatively, the functional molecules can be used without wasting them. In the case where a conventional method is used, since there are a large number of steps, functional molecules cannot be fully incorporated into PNA, and it was therefore found that the method proposed here is a method having a very good cost-performance ratio.

However, the method proposed here cannot always be said to be a perfect synthetic method. For example, when a functional molecule containing a sulfonyl chloride such as dabsyl-Cl or dansyl-Cl was reacted with Gly–^{Boc}PNA–OH (**20**), a reaction with the free amino group proceeded, but the Boc group was removed by an acid such as **54**, and the target functionalized PNA monomer unit could not be synthesized (Scheme 4).

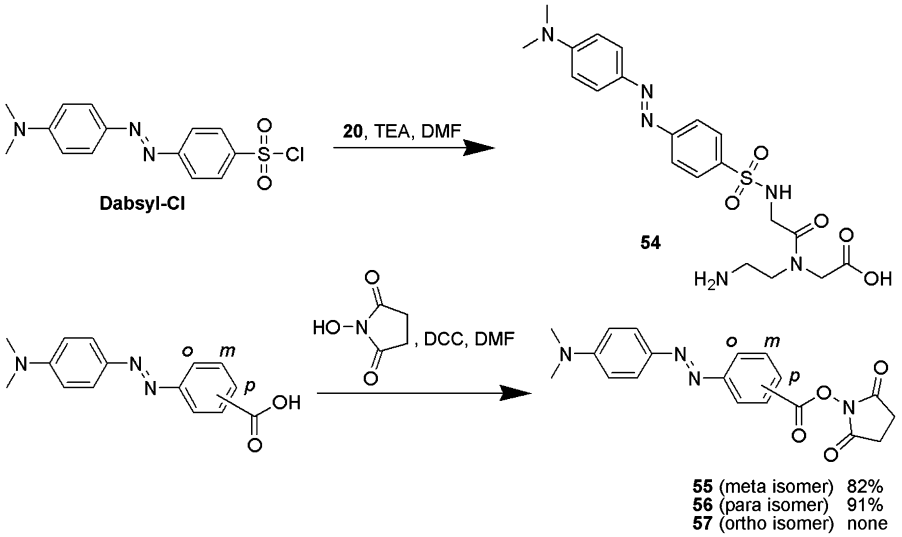
When synthesis of *ortho*-, *meta*-, and *para*-methyl red OSu derivatives was attempted in order to incorporate methyl red as the functional molecule into ω-AA–^{Boc}PNA–OH, it was found that, although the *meta*- and *para*-methyl red OSu derivatives (**55** and **56**) were obtained efficiently, the OSu derivative of *ortho*-methyl red (**57**) could not be obtained at all due to the steric hindrance of the nearest neighboring azo group. These results suggest that, when functionalized PNA monomer units cannot be synthesized efficiently by the method proposed here, although the number of steps increases, it is necessary to use the previously proposed method in which a linker is first bonded to a functional molecule, converted into its Pfp active ester derivative, and then condensed with a backbone, that is, it is necessary to select a method for the synthesis of the functionalized PNA monomer unit according to the type and structure of the functional molecule.

As described above, the use of either the previously reported method or the method reported here can successfully synthesize flavin,[8a] naphthalimide,[8b] FAM (**25**–**29**), fluorescein (**30**–**34**), dabcyl (4-([4-dimethylamino-phenyl]azo)benzoic acid) (**35**–**39**), methyl red (**40**–**44**), azobenzene (**45**–**49**), TAMRA (**50**), ROX (5(6)-carboxy-X-rhodamine) (**51**), pyrene (**52**), coumarin (**53**), etc. functionalized PNA monomer unit.[9] Furthermore, it was found that any functional molecule could be incorporated into PNA monomer units by either of these methods as long as the functional molecule contains a carboxyl group or an isothiocyanate group.

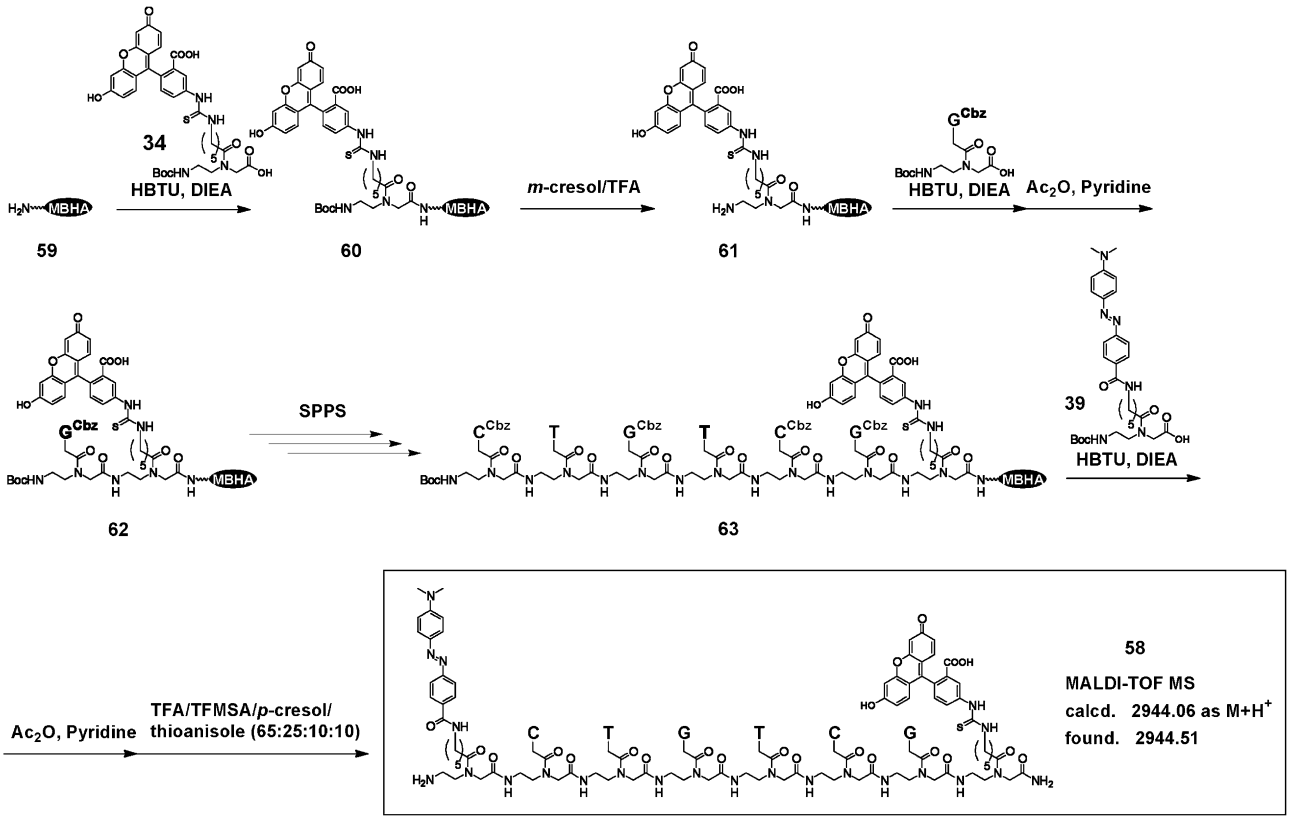
2.3. Synthesis of a functionalized PNA oligomer using functionalized PNA monomer units

We investigated whether or not functionalized PNA oligomers could be synthesized using the functionalized PNA monomer units designed above and functional molecules efficiently incorporated into the PNA oligomers at desired positions. For synthesis of the functionalized PNA oligomers, a functionalized PNA oligomer **58** into which two different types of functional molecules had been incorporated was selected as a model compound. Compounds **34** and **39** were used as the functionalized PNA monomers, and it was arranged so that they were positioned at the center, the N-terminal, and the C-terminal, respectively, of the functionalized PNA oligomer (Scheme 5).

4-Methylbenzhydrylamine resin (MBHA, **59**) was selected as the solid phase support and, following the standard solid phase *t*-Boc chemistry,[10] a PNA oligomer extension reaction was carried out. The deprotection reagent for the *t*-Boc group was 5% *m*-cresol in TFA (synthesis of **61**), HBTU (*O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium



Scheme 4.



Scheme 5. Preparation of a functionalized PNA oligomer **58**.

hexafluorophosphate)/DIEA was used as the monomer unit condensing agent (synthesis of **60** and **62**), and a mixture of acetic anhydride/pyridine/DMF (1:25:25) was used as the capping reagent (synthesis of **62**). Each reaction was followed using ninhydrin reagent, and it was confirmed that each unit condensed quantitatively. Finally, cleavage of the PNA oligomer from the solid phase support (**63**) and deprotection of the Cbz group were carried out simultaneously using TFA/TFMSA (trifluoromethanesulfonic acid)/*p*-cresol/thioanisole (60:25:10:10) to give the target **58**. The molecular weight of **58**, purified by HPLC, was determined by MALDI TOF-MS (matrix-assisted laser desorption/ionisation-time of flight mass spectrometry).

From the foregoing, a new efficient method for the synthesis of functionalized PNA oligomers, in which a functional molecule can be incorporated at a predetermined position of a PNA oligomer using the functionalized PNA monomer units reported here, has been successfully established.

3. Conclusion

PNA monomer units (ω-AA–^{Boc}PNA–OH, **20**–**24**) have been designed that enable functional molecules to be incorporated efficiently. Their use has enabled various types of functionalized PNA monomer units **25**–**53**, which could not be synthesized by conventional methods, synthesized efficiently. Furthermore, the use of functionalized PNA monomer units has enabled functional molecules to be incorporated quantitatively and at high speed at multiple positions of a PNA oligomer. As shown in the results above, an efficient method for the synthesis of functionalized PNA oligomers has been established. This has enabled PNA oligomers to be double-labeled with functional molecules, which is not reported till to date. This suggests that fluorescently labeled PNA probes having a characteristic function such as membrane permeability or organ selectivity can be designed. This is very interesting since the defect of conventional fluorescent DNA probes that they are susceptible to enzyme decomposition can be overcome by forming PNA and, in addition, another function can be imparted. Furthermore, it becomes possible to design double or multiple fluorescent PNA oligomers. As is clear from the fact that fluorescent PNA probes are already contract synthesized, it might have been unnecessary to establish this synthetic technique for designing single fluorescent PNA oligomers. However, this technique is essential for incorporating fluorescent compounds into PNA monomer units for fluorescent labeling at two or more sites. This technique is expected to play a very important role in designing molecular beacon PNA oligomers. Currently, the two types of functionalized PNA oligomers above have been synthesized and their functions are under investigation.

4. Experimental

4.1. General method

^{Boc}PNA monomer units for G(Cbz), A(Cbz), C(Cbz), and T were obtained from Applied Biosystems. ω-Amino acids

Gly–OH, β-Ala–OH, GABA–OH, and C5–OH were purchased from Watanabe Chemical Ind., as well as MBHA resin.

All reactions were carried out under a nitrogen atmosphere using dried disposable syringes and glassware when appropriate. Thin-layer chromatography (TLC) was performed on precoated plates. Products were visualized by UV light (260 nm). Flash chromatography was carried out using silica gel. Analytical and preparative HPLC were performed at room temperature. Buffer A was 0.1% TFA in water and buffer B was 0.1% TFA in acetonitrile. A linear gradient of 0–50% buffer B over 50 min at a flow rate of 10 mL/min was used. NMR spectra were recorded at 600 MHz for ^{1}H and 150 MHz for ^{13}C, respectively. ^{1}H and ^{13}C chemical shifts were given in parts per million relative to internal standard, tetramethylsilane (TMS). *J* values are given in hertz (Hz). Mixtures of two rotamers were observed for some of the products containing amide bonds. As a consequence, several of the NMR signals for these products were doubled in the rotamer ratio as indicated by 'ma' for major and 'mi' for minor. MALDI-TOF mass spectra were acquired in positive, linear mode and obtained with 2′,3′,4′-trihydroxyacetophenone as a matrix using bradykinin ([(M−1)$^{+}$] 1059.24) and oxidized insulin chain β ([(M−1)$^{+}$] 3494.96) as internal standards.

4.1.1. General procedure for the preparation of ethyl *N*-(2-(benzyloxycarbonylamino)acetyl)-*N*-(2-((*tert*-butoxy)-carbonylamino)ethyl)glycinate (Cbz–Gly–^{Boc}PNA–OEt, 9). To a solution of Cbz–Gly–OH (4.1 g, 60 mmol), ^{Boc}PNA–OEt[6] (7.0 g, 30 mmol), and TEA (triethylamine) (8.7 mL, 63 mmol) in DMF (20 mL) was added WSC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide) (6.8 g, 34.5 mmol) at 0 °C and the reaction mixture was stirred at room temperature for 15 h. To the reaction mixture was added EtOAc (200 mL), and the solution was successively washed with 5% aqueous $NaHCO_3$, 5% aqueous citric acid, and brine (each 200 mL). The organic layer was dried over $MgSO_4$ and evaporated in vacuo. The residue was flash-chromatographed (1–5% MeOH/CH_2Cl_2) to give **9** (6.3 g, 86%) as a white powder. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30–7.20 (m, 5H), 5.77 (mi) and 5.68 (ma) (br t, 1H), 5.40 (mi) and 4.97 (ma) (br t, 1H), 5.25 (mi) and 5.07 (ma) (s, 2H), 4.16 (ma) and 4.08 (mi) (m, 2H), 4.05 (ma) and 3.89 (mi) (br d, *J*=3.9 Hz, 2H), 4.00 (s, 2H), 3.48 (mi) and 3.38 (ma) (br t, 2H), 3.23 (br t, 2H), 1.38 (s, 9H), 1.25 (ma) and 1.21 (mi) (t, *J*=7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.51 (d), 169.00 (d), 156.11, 155.85, 136.36 (d), 128.43, 128.40, 128.05, 127.98, 127.96, 127.90, 79.58 (d), 66.82 (d), 61.81 (d), 48.97 (d), 48.39 (d), 42.41 (d), 38.48, 28.27, 14.03; FABMS *m*/*z* 438 [(M+H)$^{+}$].

4.1.2. Ethyl *N*-(3-(benzyloxycarbonylamino)propionyl)-*N*-(2-((*tert*-butoxy)carbonylamino)ethyl)glycinate (Cbz–β-Ala–^{Boc}PNA–OEt, 10). The compound **10** was obtained in 86% yield as a white powder in the same way as for the preparation of **9**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.35–7.15 (m, 5H), 5.60 (ma) and 5.17 (mi) (br t, 1H), 5.44 (ma) and 5.07 (mi) (br t, 1H), 5.11 (s, 2H), 4.24 (br q, 2H), 4.05 (mi) and 4.01 (ma) (br s, 2H), 3.53 (ma) and 3.48 (mi) (m, 4H), 3.27 (br s, 2H), 2.62 (ma) and 2.46 (mi) (br s, 2H), 1.45 (s, 9H), 1.32 (br t, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.62 (d), 169.66 (d), 156.36 (d), 155.97 (d), 136.59 (d), 128.39, 127.94, 79.45 (d), 66.46 (d), 61.67 (d), 49.74 (d), 48.19 (d), 38.70, 36.65, 32.93 (d), 28.29, 14.04; FABMS *m*/*z* 452 [(M+H)$^{+}$].

4.1.3. Ethyl *N*-(4-(benzyloxycarbonylamino)butanoyl)-*N*-(2-((*tert*-butoxy)carbonylamino)ethyl)glycinate (Cbz–GABA–^{Boc}PNA–OEt, 11). The compound **11** was obtained in 84% yield as a white powder in the same way as for the preparation of **9**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.3–7.2 (m, 5H), 5.41 (ma) and 5.16 (mi) (br t, 1H), 5.27 (ma) and 5.05 (mi) (br t, 1H), 5.21 (mi) and 5.00 (ma) (s, 2H), 4.10 (ma) and 4.03 (mi) (q, *J*=7.2 Hz, 2H), 3.94 (mi) and 3.88 (ma) (s, 2H), 3.40 (mi) and 3.36 (ma) (br t, *J*=5.6 Hz, 2H), 3.14 (m, 4H), 2.35 (ma) and 2.15 (mi) (t, *J*=6.5 Hz, 2H), 1.78 (m, 2H), 1.34 (s, 9H), 1.20 (mi) and 1.17 (ma) (t, *J*=7.3 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.64 (d), 169.78 (d), 156.46 (d), 155.99 (d), 136.62 (d), 128.31, 128.30, 127.93, 127.89, 127.83, 79.25 (d), 66.33 (d), 61.48 (d), 49.85 (d), 48.23 (d), 40.31 (d), 38.72, 29.71, 28.91 (d), 24.76 (d), 13.99; FABMS *m*/*z* 466 [(M+H)$^{+}$].

4.1.4. Ethyl *N*-(6-(benzyloxycarbonylamino)pentanoyl)-*N*-(2-((*tert*-butoxy)carbonylamino)ethyl)glycinate (Cbz–C4–^{Boc}PNA–OEt, 12). The compound **12** was obtained in 88% yield as a white powder in the same way as for the preparation of **9**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.4–7.3 (m, 5H), 5.45 (ma) and 5.16 (mi) (br t, 1H), 5.09 (s, 2H), 5.04 (mi) and 5.00 (ma) (br t, 1H), 4.23 (mi) and 4.20 (ma) (q, *J*=7.1 Hz, 2H), 4.04 (mi) and 3.97 (ma) (br s, 2H), 3.51 (mi) and 3.47 (ma) (br t, *J*=5.7 Hz, 2H), 3.25 (m, 2H), 3.22 (m, 2H), 2.39 (ma) and 2.22 (mi) (br t, *J*=6.8 Hz, 2), 1.75–1.60 (m, 4H), 1.57 (m, 2H), 1.42 (s, 9H), 1.32 (mi) and 1.31 (ma) (t, *J*=7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.73 (d), 169.81 (d), 159.35 (d), 155.98 (d), 136.61, 128.28, 127.85, 127.80, 79.18 (d), 66.32 (d), 61.42 (d), 49.66 (d), 48.02 (d), 40.60 (d), 37.48 (d), 31.96 (d), 30.21 (d), 28.22, 21.61 (d), 13.96; FABMS *m*/*z* 480 [(M+H)$^{+}$].

4.1.5. Ethyl *N*-(6-(benzyloxycarbonylamino)hexanoyl)-*N*-(2-((*tert*-butoxy)carbonylamino)ethyl)glycinate (Cbz–C5–^{Boc}PNA–OEt, 13). The compound **13** was obtained in 79% yield as a white powder in the same way as for the preparation of **9**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.4–7.3 (m, 5H), 5.50 (ma) and 5.14 (mi) (br t, 1H), 5.03 (ma) and 4.87 (mi) (br t, 1H), 5.30 (mi) and 5.09 (ma) (s, 2H), 4.21 (mi) and 4.17 (ma) (q, *J*=7.1 Hz, 2H), 4.03 (mi) and 3.96 (ma) (br s, 2H), 3.48 (m, 2H), 3.25 (br q, *J*=4.5 Hz, 2H), 3.17 (br t, *J*=6.4 Hz, 2H), 2.35 (ma) and 2.18 (mi) (t, *J*=7.2 Hz, 2H), 1.64, 1.51, and 1.34 (each m, 2H), 1.41 (s, 9H), 1.28 (ma) and 1.25 (mi) (t, *J*=7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.73 (d), 169.81 (d), 156.35, 155.98 (d), 136.60, 128.31, 127.89, 127.85, 79.18 (d), 66.32, 61.42 (d), 49.73 (d), 48.00 (d), 40.60 (d), 38.70, 32.39 (d), 29.47 (d), 28.24, 26.10 (d), 24.24 (d), 13.95; FABMS *m*/*z* 494 [(M+H)$^{+}$].

4.1.6. General procedure for the preparation of *N*-(2-(benzyloxycarbonylamino)acetyl)-*N*-(2-((*tert*-butoxy)-carbonylamino)ethyl)glycine (Cbz–Gly–^{Boc}PNA–OH, 14). To a solution of **9** (4.0 g, 10 mmol) in MeOH (20 mL) was added 1 N aqueous NaOH (20 mL, 20 mmol) and the reaction mixture was stirred for 3 h. The reaction mixture was

evaporated to remove MeOH, water (50 mL) was added, and the solution was washed with CH_2Cl_2 (50 mL×2). The aqueous layer was cooled down to 0 °C and adjusted to pH 3.0 using 1 N aqueous HCl. The precipitate was extracted with EtOAc (25 mL×2) and the combined organic layer was washed with brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was flash-chromatographed (5% MeOH/CH_2Cl_2) to give **14** (3.09 g, 82%) as a white powder. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.4–7.2 (m, 6H), 6.84 (ma) and 6.72 (mi) (br t, 1H), 5.03 (s, 2H), 4.11 (mi) and 3.94 (ma) (br s, 2H), 3.92 (ma) and 3.77 (mi) (br s, 2H), 3.33 (ma) and 3.29 (mi) (m, 2H), 3.09 (ma) and 3.02 (mi) (m, 2H), 1.37 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.94 (d), 169.23 (d), 156.42 (d), 155.66 (d), 137.14, 128.34, 127.77, 127.67, 77.90 (d), 65.38, 47.43 (d), 46.76 (d), 41.72 (d), 37.97 (d), 28.19 (d); HRMS (FAB$^+$) calcd for $C_{19}H_{28}N_3O_7$ [(M+H)$^+$] 410.1927, observed 410.1926.

4.1.7. *N*-(3-(Benzyloxycarbonylamino)propionyl)-*N*-(2-((*tert*-butoxy)carbonylamino)ethyl)glycine (Cbz–β-Ala–^{Boc}PNA–OH, 15). The compound **15** was obtained in 82% yield as a white powder in the same way as for the preparation of **14**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.40–7.25 (m, 5H), 7.16 (m, 1H), 6.89 (ma) and 6.86 (mi) (br t, 1H), 5.04 (mi) and 4.99 (ma) (d, J=5.3 Hz, 2H), 3.88 (br s, 2H), 3.31 (ma) and 3.26 (ma) (br t, J=6.0 Hz, 2H), 3.25–3.15 (m, 4H), 3.05 (ma) and 3.00 (mi) (br q, 2H), 1.35 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.70 (d), 171.48 (d), 156.61 (d), 156.25 (d), 136.41 (d), 128.32, 127.88, 127.36, 126.88, 80.44 (d), 66.74 (d), 49.53 (d), 47.95 (d), 39.16 (d), 37.01 (d), 32.79, 28.06; HRMS (FAB$^+$) calcd for $C_{20}H_{30}N_3O_5$ [(M+H)$^+$] 424.2084, observed 424.2082.

4.1.8. *N*-(4-(Benzyloxycarbonylamino)butanoyl)-*N*-(2-((*tert*-butoxy)carbonylamino)ethyl)glycine (Cbz–GABA–^{Boc}PNA–OH, 16). The compound **16** was obtained in 88% yield as a white powder in the same way as for the preparation of **14**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36–7.26 (m, 5H), 7.22 (br t, 1H), 6.80 (ma) and 6.69 (mi) (br t, 1H), 5.00 (s, 2H), 4.04 (mi) and 3.90 (ma) (s, 2H), 3.34 (ma) and 3.28 (ma) (br t, 2H), 3.07 (mi) and 3.01 (ma) (m, 4H), 2.33 (ma) and 2.17 (mi) (br t, 2H), 1.64 (m, 2H), 1.36 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.89 (d), 171.71 (d), 157.02 (d), 156.35 (d), 136.64, 128.43, 127.06, 80.68 (d), 66.80 (d), 50.11 (d), 48.28 (d), 40.28 (d), 38.78, 29.68 (t), 28.33, 24.95 (d); HRMS (FAB$^+$) calcd for $C_{21}H_{32}N_3O_5$ [(M+H)$^+$] 438.2240, observed 438.2238.

4.1.9. *N*-(6-(Benzyloxycarbonylamino)pentanoyl)-*N*-(2-((*tert*-butoxy)carbonylamino)ethyl)glycine (Cbz–C4–^{Boc}PNA–OH, 17). The compound **17** was obtained in 86% yield as a white powder in the same way as for the preparation of **14**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.4–7.25 (m, 5H), 7.04 (ma) and 6.31 (mi) (br t, 1H), 5.39 (ma) and 5.16 (mi) (br t, 1H), 5.08 (s, H), 4.01 (ma) and 3.88 (mi) (br s, 2H), 3.55–3.35 (m, 2H), 3.3–3.1 (m, 4H), 2.45–2.15 (m, 2H), 1.66 (m, 2H), 1.49 (m, 2H), 1.49 (mi) and 1.42 (ma) (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.86 (d), 170.97 (d), 155.33 (d), 154.64 (d), 135.14 (d), 126.70, 126.24, 78.76, 64.99, 47.97 (d), 46.30 (d), 38.79 (m), 37.70 (d), 30.67 (d), 28.67 (d), 26.62, 20.14 (d); HRMS (FAB$^+$) calcd for $C_{22}H_{34}N_3O_7$ [(M+H)$^+$] 452.2397, observed 452.2397.

4.1.10. *N*-(6-(Benzyloxycarbonylamino)hexanoyl)-*N*-(2-((*tert*-butoxy)carbonylamino)ethyl)glycine (Cbz–C5–^{Boc}PNA–OH, 18). The compound **18** was obtained in 87% yield as a white powder in the same way as for the preparation of **14**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.4–7.2 (m, 5H), 5.57 (ma) and 5.33 (mi) (br t, 1H), 5.13 (mi) and 5.08 (ma) (br s, 2H), 4.00 (ma) and 3.92 (mi) (br s, 2H), 3.49 (mi) and 3.44 (ma) (br s, 2H), 3.23 (mi) and 3.17 (ma) (m, 4H), 2.5–2.15 (m, 2H), 1.65 (m, 2H), 1.49 (br s, 2H), 1.42 (m, 11H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.21 (d), 171.72 (d), 157.06 (d), 156.58 (d), 136.41 (d), 128.36, 127.84 (d), 80.38 (d), 66.68 (d), 47.70 (d), 47.96 (d), 40.91 (d), 39.37 (d), 32.39 (d), 29.39, 28.28, 26.04, 24.34 (d); HRMS (FAB$^+$) calcd for $C_{23}H_{36}N_3O_7$ [(M+H)$^+$] 466.2553, observed 466.2548.

4.1.11. 1-(2-((*tert*-Butoxy)carbonylamino)ethyl)-1,4-diazaperhydroine-2,5-dione (19). A solution of **9** (7.40 g, 17 mmol) in EtOH (20 mL) was shaken in an atmosphere of hydrogen (1 atm) in the presence of 5% palladium on carbon (740 mg) at room temperature for 8 h. The reaction mixture was filtered through a Celite pad and the filtrate was evaporated in vacuo. The residue was flash-chromatographed (7–10% MeOH/CH_2Cl_2) to give **19** (3.89 g, 89%) as a white powder. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.01 (s, 1H), 4.85 (s, 1H), 4.09 (s, 2H), 4.02 (s, 2H), 3.54 (br s, 2H), 3.36 (br s, 2H), 1.43 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.19, 164.35, 156.22, 79.62, 50.30, 40.60, 45.04, 37.98, 28.31; FABMS m/z 258 [(M+H)$^+$].

4.1.12. General procedure for the preparation of *N*-(2-aminoacetyl)-*N*-(2-((*tert*-butoxy)carbonylamino)ethyl)-glycine (Gly–^{Boc}PNA–OH, 20). A solution of **14** (3.7 g, 8.5 mmol) in EtOH (10 mL) was shaken in an atmosphere of hydrogen in the presence of 5% palladium on carbon (190 mg) at room temperature for 8 h. The mixture was filtered through a Celite pad and the filtrate was evaporated in vacuo. The residue was flash-chromatographed (5–50% MeOH/CH_2Cl_2) to give **20** (2.18 g, 90%) as a white powder. ^{1}H NMR (600 MHz, D_2O) δ 4.01 (ma) and 4.50 (mi) (s, 2H), 3.93 (ma) and 3.84 (mi) (s, 2H), 3.49 (ma) and 3.31 (mi) (br t, 2H), 3.43 (mi) and 3.27 (ma) (br t, 2H), 1.43 (s, 9H); ^{13}C NMR (150 MHz, D_2O) δ 175.46 (d), 167.93 (d), 158.18 (d), 81.15 (d), 51.00 (d), 47.84 (d), 40.19 (d), 37.77 (m), 27.76 (d); HRMS (FAB$^+$) calcd for $C_{11}H_{22}N_3O_5$ [(M+H)$^+$] 276.1559, observed 276.1558.

4.1.13. *N*-(3-Aminopropionyl)-*N*-(2-((*tert*-butoxy)carbonylamino)ethyl)glycine (β-Ala–^{Boc}PNA–OH, 21). The compound **21** was obtained in 83% yield as a white powder in the same way as for the preparation of **20**. ^{1}H NMR (600 MHz, CD_3OD) δ 3.96 (mi) and 3.93 (ma) (s, 2H), 3.51 (ma) and 3.48 (mi) (br t, J=6.2 Hz, 2H), 3.27 (ma) and 3.24 (mi) (m, 4H), 2.87 (mi) and 2.70 (ma) (br t, 2H), 1.47 (s, 9H); ^{13}C NMR (150 MHz, CD_3OD) δ 176.13 (d), 172.61 (d), 158.45, 80.22 (d), 54.14, 51.63, 39.44 (d), 37.06 (d), 30.92 (d), 28.77; HRMS (FAB$^+$) calcd for $C_{12}H_{24}N_3O_5$ [(M+H)$^+$] 290.1716, observed 290.1719.

4.1.14. *N*-(4-Aminobutanoyl)-*N*-(2-((*tert*-butoxy)carbonylamino)ethyl)glycine (GABA–^{Boc}PNA–OH, 22). The compound **22** was obtained in 83% yield as a white powder in the same way as for the preparation of **20**. ^{1}H NMR

(600 MHz, CD_3OD) δ 3.94 (s, 2H), 3.51 (br t, 2H), 3.26 (m, 2H), 3.02 (m, 2H), 2.66 (mi) and 2.47 (ma) (br t, 2H), 2.00 (m, 2H), 1.47 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.91 (d), 165.20 (d), 148.90, 70.66 (d), 45.07 (d), 42.09, 40.36, 30.44 (m), 21.47 (d), 19.29, 14.26 (d); HRMS (FAB^+) calcd for $C_{13}H_{26}N_3O_5$ [$(M+H)^+$] 304.1872, observed 304.1868.

4.1.15. *N*-(4-Aminopentanoyl)-*N*-(2-((*tert*-butoxy)carbonylamino)ethyl)glycine (C4–^{Boc}PNA–OH, 23). The compound **23** was obtained in 67% yield as a white powder in the same way as for the preparation of **20**. ^1H NMR (600 MHz, CD_3OD) δ 3.94 (mi) and 3.93 (ma) (s, 2H), 3.51 (m, 2H), 3.26 (t, J=6.1 Hz, 2H), 2.97 (mi) and 2.95 (ma) (t, J=6.8 Hz, 2H), 2.55 (mi) and 2.38 (ma) (br t, J=5.7 Hz, 2H), 1.75–1.65 (m, 4H), 1.52 (mi) and 1.47 (ma) (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.82 (d), 165.82 (d), 148.93, 70.62 (d), 44.94, 41.94, 40.33, 30.31 (d), 23.23 (d), 19.28, 18.47, 12.72 (d); HRMS (FAB^+) calcd for $C_{14}H_{28}N_3O_5$ [$(M+H)^+$] 318.2029, observed 318.2026.

4.1.16. *N*-(6-Aminohexanoyl)-*N*-(2-((*tert*-butoxy)carbonylamino)ethyl)glycine (C5–^{Boc}PNA–OH, 24). The compound **24** was obtained in 79% yield as a white powder in the same way as for the preparation of **20**. ^1H NMR (600 MHz, CD_3OD) δ 3.94 (mi) and 3.93 (ma) (br s, 2H), 3.52 (m, 2H), 3.26 (br t, J=5.8 Hz, 2H), 2.99 (mi) and 2.95 (ma) (t, J=7.3 Hz, 2H), 2.51 (mi) and 2.34 (ma) (t, J=6.6 Hz, 2H), 1.70 (m, 4H), 1.55–1.40 (m, 11H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.96 (d), 166.38 (d), 148.89 (d), 70.59 (d), 44.90, 41.91, 40.40, 30.47 (m), 23.60 (d), 19.29, 18.46 (d), 17.11 (d), 15.56 (d); HRMS (FAB^+) calcd for $C_{15}H_{30}N_3O_5$ [$(M+H)^+$] 332.2185, observed 332.2179.

4.1.17. General procedure for the preparation of the photoactive peptide nucleic acid monomer unit.

4.1.17.1. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(2-(3′,6′-dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthene]-5-carbonylamino)acetyl)glycine (FAM–Gly–^{Boc}PNA–OH, 25). To a solution of **20** (30.3 mg, 0.10 mmol) in DMF (5 mL) were added 5,6-FAM *N*-hydroxysuccinimide ester (50 mg, 0.11 mmol) and TEA (2.5 mL, 0.2 mmol). The reaction mixture was stirred at room temperature for 15 h. The reaction mixture was evaporated and the residue was flash-chromatographed (0–25% MeOH/CH_2Cl_2) to give **25** (69.8 mg, quant.) as a light yellow powder. Since several of the signals appeared as quadruplets due to restricted rotation around the secondary amide and due to the diastereomeric mixture of 5,6-FAM, the NMR data of **25** could not be assigned. HRMS (FAB^+) calcd for $C_{32}H_{32}N_3O_{11}$ [$(M+H)^+$] 634.2037, observed 634.2034.

4.1.17.2. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(3-(3′,6′-dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthene]-5-carbonylamino)propionyl)glycine (FAM–β-Ala–^{Boc}PNA–OH, 26). The compound **26** was obtained quantitatively as a yellow powder in the same way as for the preparation of **25**. HRMS (FAB^+) calcd for $C_{33}H_{34}N_3O_{11}$ [$(M+H)^+$] 648.2193, observed 648.2191.

4.1.17.3. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(4-(3′,6′-dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthene]-5-carbonylamino)butanoyl)glycine (FAM–GABA–^{Boc}PNA–OH, 27). The compound **27** was obtained quantitatively as a yellow powder in the same way as for the preparation of **25**. HRMS (FAB^+) calcd for $C_{34}H_{36}N_3O_{11}$ [$(M+H)^+$] 662.2350, observed 662.2350.

4.1.17.4. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(4-(3′,6′-dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthene]-5-carbonylamino)pentanoyl)glycine (FAM–C4–^{Boc}PNA–OH, 28). The compound **28** was obtained quantitatively as a yellow powder in the same way as for the preparation of **25**. HRMS (FAB^+) exact mass 665.1909 $(M+H)^+$, calcd for $C_{32}H_{33}N_4O_{10}S$ 665.1917.

4.1.17.5. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(6-(3′,6′-dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthene]-5-carbonylamino)hexanoyl)glycine (FAM–C5–^{Boc}PNA–OH, 29). The compound **29** was obtained quantitatively as a yellow powder in the same way as for the preparation of **25**. HRMS (FAB^+) calcd for $C_{36}H_{40}N_3O_{11}$ [$(M+H)^+$] 690.2663, observed 690.2659.

4.1.18. General procedure for the preparation of the photoactive peptide nucleic acid monomer unit.

4.1.18.1. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(2-[[[(3′,6′-dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthen]-5-yl)amino]thioxomethyl]amino]acetyl)glycine (fluorescein–Gly–^{Boc}PNA–OH, 30). To a solution of **20** (30.3 mg, 0.10 mmol) in DMF (5 mL) were added 5,6-FAM *N*-hydroxysuccinimide ester (50 mg, 0.11 mmol) and TEA (2.5 mL, 0.2 mmol). The reaction mixture was stirred at room temperature for 15 h. The reaction mixture was evaporated and the residue was flash-chromatographed (0–25% MeOH/CH_2Cl_2) to give **30** (69.8 mg, quant.) as a light yellow powder. The NMR data of **30** could not be assigned. HRMS (FAB^+) calcd for $C_{32}H_{33}N_4O_{10}S$ [$(M+H)^+$] 665.1917, observed 665.1909.

4.1.18.2. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(3-[[[(3′,6′-dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthen]-5-yl)amino]thioxomethyl]amino]propionyl)glycine (fluorescein–β-Ala–^{Boc}PNA–OH, 31). The compound **31** was obtained in 83% yield as a yellow powder in the same way as for the preparation of **30**. The NMR data of **31** could not be assigned. HRMS (FAB^+) calcd for $C_{33}H_{35}N_4O_{10}S$ [$(M+H)^+$] 679.2074, observed 679.2066.

4.1.18.3. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(4-[[[(3′,6′-dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthen]-5-yl)amino]thioxomethyl]amino]butanoyl)glycine (fluorescein–GABA–^{Boc}PNA–OH, 32). The compound **32** was obtained in 83% yield as a yellow powder in the same way as for the preparation of **30**. The NMR data of **32** could not be assigned. HRMS (FAB^+) calcd for $C_{34}H_{37}N_4O_{10}S$ [$(M+H)^+$] 693.2230, observed 693.2235.

4.1.18.4. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(5-[[[(3′,6′-dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthen]-5-yl)amino]thioxomethyl]amino]pentanoyl)glycine (fluorescein–C4–^{Boc}PNA–OH, 33). The compound **33** was obtained in 83% yield as a yellow powder in the same way as for the preparation of **30**. The NMR data of **33** could not be assigned. HRMS (FAB^+) calcd for $C_{35}H_{39}N_4O_{10}S$ [$(M+H)^+$] 707.2387, observed 707.2393.

4.1.18.5. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(6-[[[(3′,6′-dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthen]-5-yl)amino]thioxomethyl]amino]hexanoyl)-glycine (fluorescein–C5–^{Boc}PNA–OH, 34). The compound **34** was obtained in 83% yield as a yellow powder in the same way as for the preparation of **30**. The NMR data of **34** could not be assigned. HRMS (FAB$^+$) calcd for $C_{36}H_{41}N_4O_{10}S$ [(M+H)$^+$] 721.2543, observed 721.2543.

4.1.19. General procedure for the preparation of the photoactive peptide nucleic acid monomer unit.

4.1.19.1. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(2-(4-(4′-dimethylaminophenylazo)benzoylamino)acetyl)-glycine (dabcyl–Gly–^{Boc}PNA–OH, *p*-MR–Gly–^{Boc}PNA–OH, 35). To a solution of **20** (100 mg, 0.39 mmol) in DMF (10 mL) were added dabcyl *N*-hydroxysuccinimide ester (145 mg, 0.40 mmol) and TEA (6.0 mL, 0.45 mmol). The reaction mixture was stirred at room temperature for 15 h. The reaction mixture was evaporated and the residue was flash-chromatographed (0–4% MeOH/CH_2Cl_2) to give **35** (184 mg, 91%) as a reddish brown powder. ^{1}H NMR (600 MHz, CD_3OD) δ 7.98 (d, J=8.3 Hz, 2H), 7.83 (br d, J=8.7 Hz, 2H), 7.80 (d, J=8.3 Hz, 2H), 6.80 (d, J=9.4 Hz, 2H), 4.35 (ma) and 4.20 (mi) (s, 2H), 4.06 (mi) and 3.92 (ma) (s, 2H), 3.55–3.45 (m, 2H), 3.30–3.20 (m, 2H), 3.09 (mi) and 3.09 (mi) (s, 6H), 1.44 (mi) and 1.42 (ma) (s, 9H); ^{13}C NMR (150 MHz, CD_3OD) δ 171.73, 168.94 (d), 166.80, 158.41, 156.63 (d), 154.70, 144.89, 129.86, 129.53 (d), 126.43, 123.00, 112.64, 80.38 (d), 69.15, 42.58 (d), 40.29 (d), 38.41 (d), 28.75 (d), 26.28; HRMS (FAB$^+$) calcd for $C_{26}H_{34}N_6O_6$ [M$^+$] 526.2540, observed 526.2525.

4.1.19.2. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(3-(4-(4′-dimethylaminophenylazo)benzoylamino)propionyl)glycine (dabcyl–β-Ala–^{Boc}PNA–OH, *p*-MR–β-Ala–^{Boc}PNA–OH, 36). The compound **36** was obtained in 89% yield as a reddish brown powder in the same way as for the preparation of **35**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.57 (mi) and 8.53 (ma) (br t, 1H), 7.97 (m, 2H), 7.81 (m, 1H), 6.85 (d, J=8.6 Hz, 2H), 4.05 (mi) and 3.94 (ma) (s, 2H), 3.48 (m, 2H), 3.37 (m, 2H), 3.17 (mi) and 3.08 (ma) (s, 6H), 2.65 (m, 2H), 1.36 (mi) and 1.34 (ma) (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.01 (d), 171.09 (d), 165.50, 155.59 (d), 153.91, 152.81, 142.62, 134.63 (d), 128.21 (d), 125.03, 121.44, 111.54, 77.72 (d), 48.06 (d), 38.02 (d), 35.93 (d), 31.93 (d), 28.15 (d), 25.18; HRMS (FAB$^+$) calcd for $C_{27}H_{36}N_6O_6$ [M$^+$] 540.2696, observed 540.2704.

4.1.19.3. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(4-(4-(4′-dimethylaminophenylazo)benzoylamino)butanoyl)glycine (dabcyl–GABA–^{Boc}PNA–OH, *p*-MR–GABA–^{Boc}PNA–OH, 37). The compound **37** was obtained in 85% yield as a reddish brown powder in the same way as for the preparation of **35**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.56 (br t, J=4.9 Hz, 1H), 7.98 (d, J=8.3 Hz, 2H), 7.81 (t, J=9.1 Hz, 4H), 6.85 (d, J=9.1 Hz, 2H), 4.07 (mi) and 3.92 (ma) (s, 2H), 3.4–3.2 (m, 4H), 3.08 (s, 6H), 2.42 (ma) and 2.25 (mi) (br t, J=6.8 Hz, 2H), 1.79 (m, 2H), 1.36 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 174.35, 170.95, 165.58, 160.18 (d), 153.86, 152.80, 142.60, 135.34 (d), 128.26, 125.02, 121.41, 111.54, 78.77, 48.18, 40.96, 39.87, 30.43, 28.15, 24.63 (d), 23.47; HRMS (FAB$^+$) calcd for $C_{28}H_{38}N_6O_6$ [M$^+$] 554.2853, observed 554.2863.

4.1.19.4. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(6-(4-(4′-dimethylaminophenylazo)benzoylamino)pentanoyl)glycine (dabcyl–C4–^{Boc}PNA–OH, *p*-MR–C4–^{Boc}PNA–OH, 38). The compound **38** was obtained in 98% yield as a reddish brown powder in the same way as for the preparation of **35**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.62 (ma) and 8.58 (mi) (br t, 1H), 7.99 (t, J=8.6 Hz, 2H), 7.80 (m, 4H), 6.84 (d, J=7.8 Hz, 2H), 3.77 (mi) and 3.72 (ma) (s, 2H), 3.4–3.2 (m, 6H), 3.07 (s, 6H), 2.33 (mi) and 2.21 (ma) (br t, 2H), 1.6–1.5 (m, 4H), 1.35 (mi) and 1.34 (ma) (br s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.96 (d), 172.28 (d), 165.46, 155.56, 153.86, 152.79, 142.63, 134.93 (d), 128.26 (d), 125.03, 121.43, 111.54, 77.55 (d), 47.23 (d), 38.00 (d), 31.74 (d), 28.81 (d), 28.22, 25.18, 22.18 (d); HRMS (FAB$^+$) calcd for $C_{29}H_{41}N_6O_6$ [(M+H)$^+$] 569.3088, observed 569.3083.

4.1.19.5. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(6-(4-(4′-dimethylaminophenylazo)benzoylamino)hexanoyl)glycine (dabcyl–C5–^{Boc}PNA–OH, *p*-MR–C5–^{Boc}PNA–OH, 39). The compound **39** was obtained in 98% yield as a reddish brown powder in the same way as for the preparation of **35**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.54 (m, 1H), 7.96 (d, J=8.3 Hz, 2H), 7.82 (d, J=8.6 Hz, 2H), 7.76 (d, J=6.1 Hz, 2H), 6.85 (d, J=9.1 Hz, 2H), 4.10 (mi) and 3.94 (ma) (s, 2H), 3.48 (m, 2H), 3.37 (br t, J=5.7 Hz, 2H), 3.08 (s, 6H), 3.04 (m, 4H), 2.65 (br t, J=7.2 Hz, 2H), 1.37 (mi) and 1.34 (ma) (br s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.98 (d), 170.90 (d), 165.49, 155.57, 153.90, 152.80, 142.61, 134.63 (d), 128.19, 125.02, 121.43, 111.54, 77.73 (d), 47.37 (d), 40.96, 38.00 (d), 35.98, 31.96 (d), 28.17, 28.09, 25.17; HRMS (FAB$^+$) calcd for $C_{30}H_{42}N_6O_6$ [M$^+$] 582.3166, observed 582.3170.

4.1.20. General procedure for the preparation of the photoactive peptide nucleic acid monomer unit.

4.1.20.1. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(2-(3-(4′-dimethylaminophenylazo)benzoylamino)acetyl)-glycine (*m*-MR–Gly–^{Boc}PNA–OH, 40). To a solution of **20** (50 mg, 0.18 mmol) in DMF (10 mL) were added *m*-MR–OSu (73 mg, 0.20 mmol) and TEA (3.5 mL, 0.27 mmol). The reaction mixture was stirred at room temperature for 15 h. The reaction mixture was evaporated and the residue was flash-chromatographed (0–10% MeOH/CH_2Cl_2) to give **40** (95 mg, quant.) as an orange powder. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.26 (s, 1H), 7.92 (d, J=7.6 Hz, 2H), 7.83 (d, J=9.1 Hz, 2H), 7.62 (t, J=7.6 Hz, 1H), 6.88 (ma) and 6.74 (mi) (br t, 1H), 6.85 (d, J=9.1 Hz, 2H), 4.22 (d, J=2.7 Hz, 2H), 3.99 (ma) and 3.89 (mi) (s, 2H), 3.44 (t, J=6.4 Hz, 1H), 3.4–3.25 (br s, 4H), 3.07 (s, 6H), 1.39 (ma) and 1.37 (mi) (s, 9H); ^{13}C NMR (150 MHz, CD_3OD) δ 175.68 (d), 171.49 (d), 169.61 (d), 158.34 (d), 154.50 (d), 154.41 (d), 144.59, 135.89 (d), 130.33 (d), 129.10 (d), 126.24, 122.19 (d), 112.58, 80.30 (d), 69.10, 42.59 (d), 40.39, 39.37 (d), 28.78 (d), 26.23; HRMS (FAB$^+$) calcd for $C_{26}H_{35}N_6O_6$ [(M+H)$^+$] 527.2618, observed 527.2614.

4.1.20.2. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(3-(3-(4′-dimethylaminophenylazo)benzoylamino)propionyl)glycine (*m*-MR–β-Ala–^{Boc}PNA–OH, 41). The compound **41** was obtained in 89% yield as an orange powder in the same way as for the preparation of **40**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.90 (ma) and 7.87 (mi)

(d, J=7.6 Hz, 1H), 7.86 (d, J=8.7 Hz, 2H), 7.80 (ma) and 7.77 (mi) (d, J=7.5 Hz, 1H), 7.49 (t, J=7.9 Hz, 1H), 6.74 (d, J=8.7 Hz, 2H), 4.12 (mi) and 4.03 (ma) (br s, 2H), 3.74 (m, 2H), 3.49 (br t, 2H), 3.24 (br t, 2H), 3.08 (s, 6H), 1.43 (mi) and 1.37 (ma) (br s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.70, 171.53 (d), 168.24 (d), 156.35 (d), 152.94, 152.84, 143.26, 134.93 (d), 129.31, 128.03 (d), 125.36, 125.06, 120.56 (d), 111.60, 79.89 (d), 49.19 (d), 40.28, 38.63 (d), 36.19 (d), 32.35 (d), 28.30, 25.33; HRMS (FAB$^+$) calcd for $C_{27}H_{37}N_6O_6$ [(M+H)$^+$] 541.2775, observed 541.2779.

4.1.20.3. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(4-(3-(4′-dimethylaminophenylazo)benzoylamino)butanoyl)glycine (*m*-MR–GABA–^{Boc}PNA–OH, 42). The compound **42** was obtained in 91% yield as an orange powder in the same way as for the preparation of **40**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.16 (ma) and 8.14 (mi) (s, 1H), 7.90 (br d, J=7.9 Hz, 1H), 7.86 (br d, 2H), 7.84 (d, J=8.6 Hz, 1H), 7.48 (t, J=7.6 Hz, 1 H), 6.73 (d, J=8.7 Hz, 2H), 4.12 (mi) and 3.98 (ma) (br s, 2H), 3.49 (m, 4H), 3.25 (m, 2H), 3.08 (mi) and 3.07 (ma) (s, 6H), 2.56 (ma) and 2.34 (mi) (br t, 2H), 1.99 (m, 2H), 1.43 (mi) and 1.38 (ma) (br s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.95 (d), 171.50 (d), 167.75 (d), 156.40 (d), 152.87, 152.66 (d), 143.17 (d), 135.08 (d), 129.31 (d), 128.43 (d), 125.41 (d), 124.19 (d), 121.16 (d), 111.61, 79.73 (d), 49.34 (d), 40.30, 39.88 (d), 38.69 (d), 30.36 (d), 28.34, 25.32, 23.72 (d); HRMS (FAB$^+$) calcd for $C_{28}H_{39}N_6O_6$ [(M+H)$^+$] 555.2931, observed 555.2928.

4.1.20.4. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(6-(3-(4′-dimethylaminophenylazo)benzoylamino)pentanoyl)glycine (*m*-MR–C4–^{Boc}PNA–OH, 43). The compound **43** was obtained in 91% yield as an orange powder in the same way as for the preparation of **40**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.68 (ma) and 8.66 (mi) (br t, 1H), 8.23 (br s, 1H), 7.91 and 7.88 (each d, J=7.7 Hz, 1H), 7.82 (br d, J=8.9 Hz, 2H), 7.58 (br t, J=7.6 Hz, 1H), 6.84 (d, J=8.9 Hz, 2H), 3.75 (mi) and 3.70 (ma) (br s, 2H), 3.5–3.2 (m, 6H), 3.06 (s, 6H), 2.32 (mi) and 2.20 (ma) (br t, 2H), 1.54 (m, 4H), 1.35 (mi) and 1.33 (ma) (br s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.96 (d), 172.24 (d), 165.60, 155.57, 152.68, 152.36, 142.51, 135.79, 128.89 (d), 128.05 (d), 124.89, 123.92 (d), 120.65 (d), 111.55, 77.54 (d), 48.23 (d), 37.99 (d), 31.77 (d), 28.81 (d), 28.23, 28.19, 25.18, 22.23 (d); HRMS (FAB$^+$) calcd for $C_{29}H_{41}N_6O_6$ [(M+H)$^+$] 569.3088, observed 569.3082.

4.1.20.5. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(6-(3-(4′-dimethylaminophenylazo)benzoylamino)hexanoyl)glycine (*m*-MR–C5–^{Boc}PNA–OH, 44). The compound **44** was obtained in 95% yield as an orange powder in the same way as for the preparation of **40**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.62 (br t, 1H), 8.23 (ma) and 8.20 (mi) (br s, 1H), 7.89 (m, 2H), 7.82 (ma) and 7.76 (mi) (d, J=9.1 Hz, 2H), 7.59 (br t, J=8.0 Hz, 1H), 6.84 (ma) and 6.68 (mi) (d, J=9.1 Hz, 2H), 3.95 (mi) and 3.88 (ma) (br s, 2H), 3.35–3.25 (m, 4H), 3.06 (s, 6H), 3.01 (m, 4H), 2.32 (ma) and 2.16 (mi) (br t, J=7.1 Hz, 2H), 1.54 (m, 4H), 1.35 (mi) and 1.33 (ma) (br s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.42 (d), 171.38 (d), 165.47, 155.45, 152.83 (d), 152.25 (d), 142.47 (d), 135.77 (d), 129.01, 127.81 (d), 124.94 (d), 123.74, 120.45 (d), 111.31 (d), 77.53 (d), 47.30 (d), 45.80 (d), 39.35 (d), 37.96 (d), 31.74 (d), 29.12 (d), 28.81 (d), 28.04 (d), 26.06, 25.07, 24.28 (d); HRMS (FAB$^+$) calcd for $C_{30}H_{43}N_6O_6$ [(M+H)$^+$] 583.3244, observed 583.3248.

4.1.21. General procedure for the preparation of the photoactive peptide nucleic acid monomer unit.

4.1.21.1. *N*-(2-(Azobenzene-4-carbonylamino)acetyl)-*N*-(2-((*tert*-butoxy)carbonylamino)ethyl)glycine (Azo–Gly–^{Boc}PNA–OH, 45). To a solution of **20** (50 mg, 0.18 mmol) in DMF (10 mL) were added Azo–OSu (73 mg, 0.20 mmol) and TEA (3.5 mL, 0.27 mmol). The reaction mixture was stirred at room temperature for 15 h. The reaction mixture was evaporated and the residue was flash-chromatographed (0–10% MeOH/CH_2Cl_2) to give **45** (95 mg, quant.) as an orange powder. ^{1}H NMR (600 MHz, CD_3OD) δ 7.95 (d, J=7.1 Hz, 2H), 7.85–7.75 (m, 4H), 7.5–7.4 (m, 3H), 4.3–4.0 (m, 2H), 3.94 (ma) and 3.90 (mi) (br d, J=9.4 Hz, 2H), 3.50–3.35 (m, 2H), 3.2–3.1 (m, 2H), 1.34 (mi) and 1.31 (ma) (s, 9H); ^{13}C NMR (150 MHz, CD_3OD) δ 175.80 (d), 171.46 (d), 169.39 (d), 158.45 (d), 157.74 (d), 155.73, 153.92 (d), 132.87, 130.33, 129.73 (d), 124.06, 123.74 (d), 80.36 (d), 69.14, 42.64 (d), 39.39 (d), 28.78, 26.24; HRMS (FAB$^+$) calcd for $C_{24}H_{30}N_5O_6$ [(M+H)$^+$] 484.2196, observed 484.2187.

4.1.21.2. *N*-(3-(Azobenzene-4-carbonylamino)propionyl)-*N*-(2-((*tert*-butoxy)carbonylamino)ethyl)glycine (Azo–β-Ala–^{Boc}PNA–OH, 46). The compound **46** was obtained in 82% yield as an orange powder in the same way as for the preparation of **45**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.71 (ma) and 8.63 (mi) (br t, 1H), 8.2–8.0 (m, 2H), 7.94 (m, 4H), 7.61 (m, 3H), 6.86 (ma) and 6.79 (mi) (br t, 1H), 3.95 (mi) and 3.92 (ma) (s, 2H), 3.50 (ma) and 3.40 (mi) (br t, 2H), 3.37 (ma) and 3.32 (mi) (br t, 2H), 3.08 (ma) and 3.05 (mi) (m, 2H), 2.65 (ma) and 2.59 (mi) (m, 2H), 1.35 (mi) and 1.34 (ma) (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.50 (d), 171.07 (d), 165.26 (d), 155.56, 153.86, 153.18 (d), 151.90, 131.96 (d), 129.50, 128.41 (d), 122.69 (d), 122.30 (d), 77.67 (d), 47.55 (d), 37.98 (d), 36.08 (d), 31.83 (d), 28.14 (d), 25.18; HRMS (FAB$^+$) calcd for $C_{25}H_{32}N_5O_6$ [(M+H)$^+$] 498.2353, observed 498.2358.

4.1.21.3. *N*-(4-(Azobenzene-4-carbonylamino)butanoyl)-*N*-(2-((*tert*-butoxy)carbonylamino)ethyl)glycine (Azo–GABA–^{Boc}PNA–OH, 47). The compound **47** was obtained in 88% yield as an orange powder in the same way as for the preparation of **45**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.00 (ma) and 8.71 (mi) (br t, 1H), 8.18 (ma) and 8.06 (mi) (d, J=7.9 Hz, 2H), 7.93 (m, 4H), 7.62 (m, 3H), 7.15–7.00 (m, 1H), 3.73 (mi) and 3.67 (ma) (s, 2H), 3.40–3.25 (m, 4H), 3.08 (mi) and 3.02 (ma) (q, J=5.3 Hz, 2H), 2.38 (mi) and 2.25 (ma) (br t, J=6.1 Hz, 2H), 1.76 (m, 2H), 1.36 (mi) and 1.34 (ma) (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.46, 171.42, 165.15 (d), 155.54, 155.50, 153.10 (d), 151.91, 136.76, 131.85, 129.48, 128.55 (d), 122.66, 122.16, 77.46 (d), 53.09, 47.78, 46.81, 29.40, 28.21, 28.16 (d), 24.63 (d); HRMS (FAB$^+$) calcd for $C_{26}H_{34}N_5O_6$ [(M+H)$^+$] 512.2509, observed 512.2516.

4.1.21.4. *N*-(6-(Azobenzene-4-carbonylamino)pentanoyl)-*N*-(2-((*tert*-butoxy)carbonylamino)ethyl)glycine (Azo–C4–^{Boc}PNA–OH, 48). The compound **48** was obtained in 92% yield as an orange powder in the same way

as for the preparation of **45**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.99 (ma) and 8.70 (mi) (br t, 1H), 8.18 (ma) and 8.06 (mi) (d, J=7.9 Hz, 2H), 7.93 (m, 4H), 7.62 (m, 3H), 7.1–7.0 (m, 1H), 3.74 (mi) and 3.67 (ma) (s, 2H), 3.4–3.2 (m, 6H), 3.07 (mi) and 3.01 (ma) (m, 2H), 2.25 (ma) and 2.19 (mi) (br t, 2H), 1.6–1.5 (m, 4H), 1.36 (mi) and 1.34 (ma) (br s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.46, 165.00, 162.25, 155.54, 153.46, 153.10 (d), 151.91, 131.88 (d), 129.49 (d), 128.54 (d), 122.66, 122.21 (d), 77.46 (d), 53.06, 46.80, 38.25 (d), 37.92 (d), 29.42, 28.18 (d), 24.63 (d); HRMS (FAB$^+$) calcd for $C_{27}H_{36}N_5O_6$ [(M+H)$^+$] 526.2666, observed 526.2675.

4.1.21.5. *N*-(6-(Azobenzene-4-carbonylamino)hexanoyl)-*N*-(2-((*tert*-butoxy)carbonylamino)ethyl)glycine (Azo–C5–^{Boc}PNA–OH, 49). The compound **49** was obtained in 87% yield as an orange powder in the same way as for the preparation of **45**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.76 (ma) and 8.68 (mi) (br s, 1H), 8.07 (br d, 2H), 7.93 (m, 4H), 7.61 (m, 3H), 6.89 (ma) and 6.86 (mi) (br t, 1H), 3.73 (mi) and 3.65 (ma) (s, 2H), 3.5–3.1 (m, 10H), 3.01 (m, 4H), 2.28 (mi) and 2.16 (mi) (br t, 2H), 1.52 (m, 4H), 1.34 (br s, 9H); HRMS (FAB$^+$) calcd for $C_{28}H_{38}N_5O_6$ [(M+H)$^+$] 540.2822, observed 540.2189.

4.1.21.6. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(2-(3′,6′-bis(dimethylamino)-3-oxospiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthene]-6-carbonylamino)acetyl)glycine (TAMRA–Gly–^{Boc}PNA–OH, 50). To a solution of **20** (5.8 mg, 21 μmol) in CH_2Cl_2 (5 mL) were added 5,6-TAMRA *N*-hydroxysuccinimide ester (5.0 mg, 9.5 μmol) and TEA (20 μL, 140 μmol). The reaction mixture was stirred at room temperature for 15 h. The reaction mixture was evaporated and the residue was flash-chromatographed (0–30% MeOH/CH_2Cl_2) to give **50** (6.0 mg, quant.) as a heliotrope powder. Since several of the signals appeared as quadruplets due to restricted rotation around the secondary amide and due to the diastereomeric mixture of 5,6-TAMRA, the NMR data of **51** could not be assigned. HRMS (FAB$^+$) calcd for $C_{36}H_{42}N_5O_9$ [(M+H)$^+$] 688.2993, observed 688.2983.

4.1.21.7. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(2-(2′,3′,6′,7′,12′,13′,16′,17′-octahydro-3-oxospiro[isobenzofuran-1(3*H*),9′-[1*H*,5*H*,9*H*,11*H*,15*H*]xantheno[2,3,4-ij:5,6,7-i′j′]diquinolizine]-5-carbonylamino)acetyl)glycine (ROX–Gly–^{Boc}PNA–OH, 51). To a solution of **20** (4.9 mg, 18 μmol) in CH_2Cl_2 (5 mL) were added 5,6-ROX *N*-hydroxysuccinimide ester (5.0 mg, 8.0 μmol) and TEA (20 μL, 140 μmol). The reaction mixture was stirred at room temperature for 15 h. The reaction mixture was evaporated and the residue was flash-chromatographed (0–30% MeOH/CH_2Cl_2) to give **51** (6.0 mg, quant.) as a purple powder. Since several of the signals appeared as quadruplets due to restricted rotation around the secondary amide and due to the diastereomeric mixture of 5,6-ROX, the NMR data of **51** could not be assigned. HRMS (FAB$^+$) exact mass 792.3615 (M+H)$^+$, calcd for $C_{32}H_{32}N_3O_{11}$ 792.3530. HRMS (FAB$^+$) calcd for $C_{44}H_{50}N_5O_9$ [(M+H)$^+$] 792.3609, observed 792.3615.

4.1.21.8. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(2-(4-(1,9-dihydropyrenyl)butanoylamino)acetyl)glycine (pyrene–Gly–^{Boc}PNA–OH, 52). To a solution of **20** (25 mg, 90 μmol) in DMF (5 mL) were added 1-pyrenebutyric acid *N*-hydroxysuccinimide ester (39 mg, 100 μmol) and TEA (138 μL, 1.0 mmol). The reaction mixture was stirred at room temperature for 15 h. The reaction mixture was evaporated and the residue was flash-chromatographed (0–10% MeOH/CH_2Cl_2) to give **52** (30 mg, 61%) as a light yellow powder. HRMS (FAB$^+$) calcd for $C_{31}H_{35}N_3O_6Na$ [(M+Na+H)$^+$] 568.2424, observed 568.2429.

4.1.21.9. *N*-(2-((*tert*-Butoxy)carbonylamino)ethyl)-*N*-(2-(7-(diethylamino)-2-oxo-2*H*-1-benzopyran-3-carbonylamino)acetyl)glycine (coumarin–Gly–^{Boc}PNA–OH, 53). To a solution of **20** (12.7 mg, 46 μmol) in DMF (5 mL) were added 7-diethylaminocoumarin-3-carboxylic acid *N*-hydroxysuccinimide ester (15 mg, 42 μmol) and TEA (55.5 μL, 0.4 mmol). The reaction mixture was stirred at room temperature for 15 h. The reaction mixture was evaporated and the residue was flash-chromatographed (0–20% MeOH/CH_2Cl_2) to give **53** (23 mg, quant.) as a yellow powder. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.68 (ma) and 8.66 (mi) (s, 1H), 7.70 (ma) and 7.69 (mi) (d, J=9.1 Hz, 1H), 6.89 (ma) and 6.75 (mi) (br t, 1H), 6.80 (d, J=9.1 Hz, 1H), 6.62 (s, 1H), 4.25 (ma) and 4.07 (mi) (br d, 2H), 4.13 (m, 1H), 3.98 (ma) and 3.89 (mi) (s, 2H), 3.48 (q, J=6.8 Hz, 4H), 3.35 (m, 2H), 3.13 (ma) and 3.07 (mi) (br q, 2H), 1.37 (mi) and 1.36 (ma) (s, 9H), 1.14 (t, J=6.8 Hz, 6H). HRMS (FAB$^+$) calcd for $C_{31}H_{35}N_3O_6$ [(M+H)$^+$] 519.2455, observed 519.2458.

4.1.22. General procedure for the preparation of the active ester of a photoactive molecule.

4.1.22.1. 3-((4-(Dimethylamino)phenyl)azo)benzoic acid *N*-hydroxysuccinimidyl ester (*m*-MR–OSu, 55). To a solution of *m*-methyl red (*m*-MR–OH; 110 mg, 0.41 mmol) and *N*-hydroxysuccinimide (60 mg, 0.52 mmol) in DMF (7 mL) was added DCC (dicyclohexylcarbodiimide) (100 mg, 0.50 mmol) at 0 °C and the reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 15 h. The reaction mixture was filtered and the filtrate was evaporated in vacuo. The residue was flash-chromatographed (CH_2Cl_2) to give **55** (124 mg; 82%) as an orange powder. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.59 (br t, 1H), 8.13 (br t, J=9.1 Hz, 2H), 7.90 (d, J=9.1 Hz, 2H), 7.61 (t, J=7.9 Hz, 1H), 6.77 (d, J=9.1 Hz, 2H), 3.11 (s, 6H), 2.93 (br s, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.09, 161.76, 153.38, 152.90, 143.47, 130.68, 129.47, 128.36, 126.02, 125.44, 124.31, 111.49, 40.26, 25.70; HRMS (FAB$^+$) calcd for $C_{19}H_{19}N_4O_4$ [(M+H)$^+$] 367.1406, observed 367.1411.

4.1.22.2. 4-((4-(Dimethylamino)phenyl)azo)benzoic acid *N*-hydroxysuccinimidyl ester (*p*-MR–OSu, 56). The compound **56** was obtained in 91% yield as an orange powder in the same way as for the preparation of **55**.

4.2. Synthesis of functionalized PNA oligomer 58

Lowering titer of solid phase support: Following the solid phase *t*-Boc chemistry previously reported by Koch et al.,[10] a condensation reaction was first carried out with MBHA solid phase support **59** (50 mg) using functionalized PNA monomer unit **34** (5.8 mg, 10 μmol), and HBTU (4.5 mg, 15 μmol) and DIEA (6.9 μL, 20 μmol) as the condensing agent, for 2 h at room temperature (synthesis of

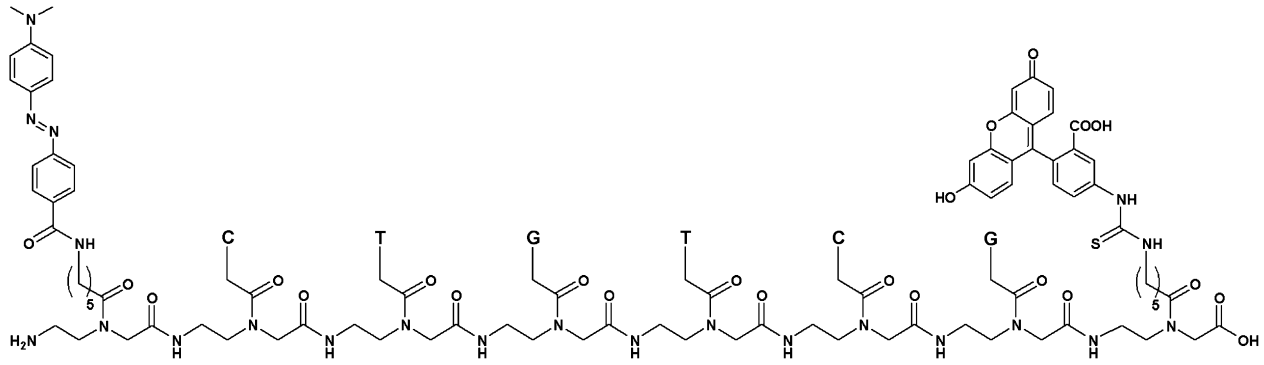


Chart 2.

60). Next, capping of unreacted amino groups of the MBHA was carried out three times using the capping reagent Ac_2O/pyridine/DMF (1:2:2) for 30 min at room temperature. Completion of the capping reaction was confirmed by ninhydrin reagent (Chart 2).

Design of base sequence recognition region: After deprotecting the Boc group by a TFA treatment (95% TFA/5% *m*-cresol) (synthesis of **61**), a condensation reaction using G(Cbz) PNA monomer unit (5.5 mg, 10 μmol), and HBTU (3.8 mg, 10 μmol) and DIEA (1.8 μL, 10 μmol) as the condensing agent was carried out on the MBHA for 30 min at room temperature (synthesis of **62**). After confirming completion of the condensation reaction with ninhydrin reagent, capping of unreacted amino groups on the MBHA was carried out using the capping reagent Ac_2O/pyridine/DMF (1:25:25) for 5 min at room temperature (synthesis of **62**). This process was repeated a further seven times using the T PNA monomer unit, C(Cbz) PNA monomer unit, G(Cbz) PNA monomer unit, and the functionalized PNA monomer unit **39** according to the sequence above (synthesis of **63**).

Cleavage from support/purification: Finally, cleavage from the solid phase support and deprotection of the Cbz were carried out simultaneously using the cleavage reagent TFA/TFMSA/*p*-cresol/thioanisole (60:25:10:10). A gradient composed of A (0.05% TFA in water) and B (0.05% TFA in acetonitrile) was used for analytical and preparative HPLC: time 0, 0% B. Time 50 min, 50% B (flow rate: 10 mL/min, detection: 260 nm). The purified PNA oligomer **58** was identified by MALDI-TOF MS. Calcd 2944.06 ($M+H^+$), found 2944.51.

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