Photolabile Peptide Nucleic Acid Monomers: Synthesis and Photodeprotection

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Received 27 February 2006

Abstract: The photolabile 2-(2-nitrophenyl)propyloxy carbonyl (NPPOC) group has been introduced as an amino protecting group for peptide nucleic acids (PNA) to be used as building blocks in photolithographic solid-phase PNA synthesis. NPPOC-protected PNA derivatives undergo fast light-promoted deprotection with high per step coupling efficiency.

Key words: solid-phase synthesis, genomics, DNA, protecting groups, photolabile

A peptide nucleic acid (PNA) is a DNA analogue in which an *N*-(2-aminoethyl)glycine polyamide structure substitutes the sugar-phosphate backbone.[1] It exhibits unique physical and chemical properties – being an achiral and uncharged biopolymer of high biological and chemical stability – and has a high but nevertheless specific binding affinity to complementary nucleic acids. For these reasons, PNA is applied as an agent in the fields of DNA-based diagnostics, therapy and biotechnology.[2]

In 1997, Weiler et al.[3] demonstrated that arrays of PNA oligomers of individual sequence could be synthesized on polymer membranes. The size of the elements in physically directed arrays is inherently limited by the volume of reagents that can be accurately delivered.[4] Solid-phase chemistry, photolabile protecting groups, and photolithography have been combined to achieve light-directed, spatially addressable parallel chemical synthesis to yield a highly diverse set of chemical products.[5] Light-directed oligonucleotide synthesis provides an efficient and versatile method for microfabricating probe arrays with densities as high as 10^6 unique sequences/cm^2. In recent years there has been growing interest in the synthesis of microarrays of oligonucleotides on glass or other surfaces utilizing photolithographic processes, for use in genomics research.[6]

Among the many factors responsible for the success of the photolithographic synthesis, are three dominant ones: the accuracy in consecutive alignment of the masks, the efficiency of removal of the photoprotecting groups and the yields of the per step coupling. The first factor is a matter of mechanical and optical instrumentation and relies on the appropriate adoption of technology originating from semiconductor chip production. Singh-Gasson et al.[7] in 1999 developed a maskless technology to direct light to specific locations. This technology uses a Digital Micro-mirror Device (DMD), which replaces the physical masks and can be conveniently controlled by a computer. Chemically, the per step coupling yields and the efficiency of photoremoval of photoprotected monomers are quality-determining factors.

The use of photolabile protecting groups in nucleic acid[8] has been well established. Useful photolabile protecting groups must be stable to mild chemical treatments, but photolytically cleaved in high yield by irradiation at wavelengths, which do not damage the protected molecule. Keeping this in view and in continuation of our work[9] on photolabile 2-(2-nitrophenyl)propyloxy carbonyl (NPPOC) groups, the synthesis of photolabile NPPOC-protected PNA monomers is described herein. Also a light-directed maskless PNA array synthesis on the glass using procedures based on photolithography and surface fluorescence is presented.

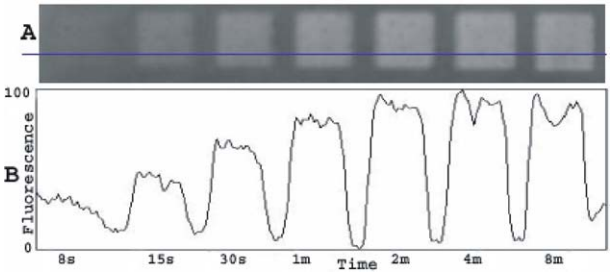
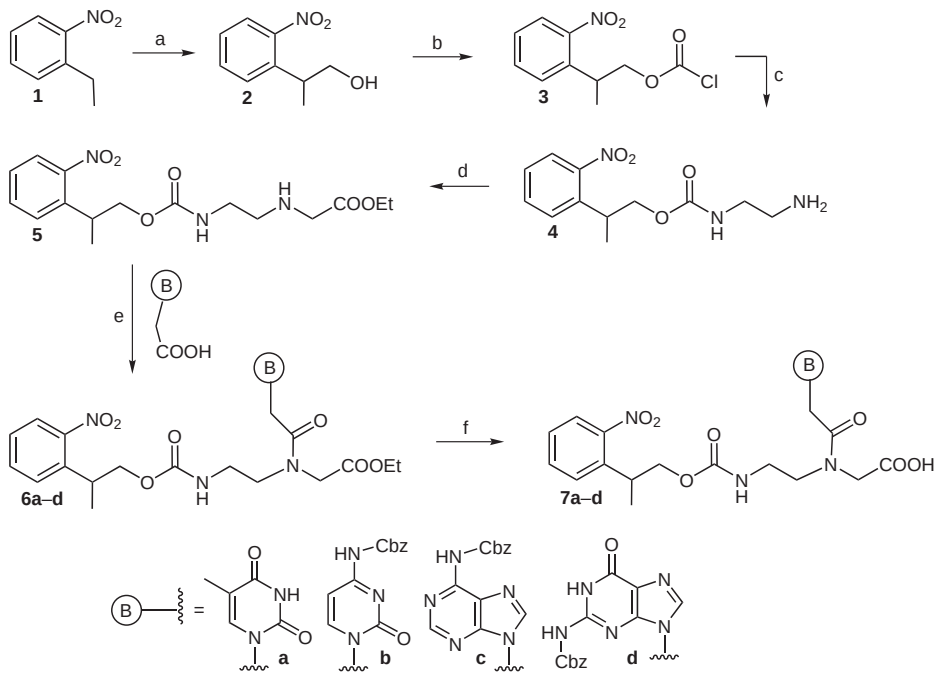


Figure 1 One layer of peptide nucleic acid coupling to amino-coated glass slide using NPPOC-T. A is the scanned slide at 532 nm excitation after fluorescent dye treatment; the brightest pixel (ca. 100 μm across) is due to UV exposure at 8 min, and the dimmest is due to 8 s UV exposure. B depicts the intensity of fluorescence at each time interval, indicating an optimum time (i.e., shortest effective time) of ca. 2 min.

To obtain NPPOC-protected PNA monomers **7a**–**d**, NPPOC-chloride[9] (**3**) was converted into NPPOC-ethane-1,2-diamine (**4**) by treatment with ethylenediamine (Scheme 1).[10] Compound **4** was reacted with ethyl bromoacetate[11] to form **5**. The amine **5** was condensed[12] with acetic acid derivative of side-chain-protected heterocyclic bases[13] **a**–**d** to give the NPPOC-protected PNA monomer esters **6a**–**d**. These were then treated with cold aqueous lithium hydroxide[14] and the acids[15] **7a**–**d** were isolated following neutralization with aqueous hydrochloric acid.

For the light-directed maskless PNA arrays synthesis, it was worthwhile to evaluate the efficacy of the critical photomediated deprotection in an array format,[16] NPPOC-T was coupled to an amino-glass slide[17] mounted

SYNLETT 2006, No. 13, pp 2130–2132
Advanced online publication: 09.08.2006
DOI: 10.1055/s-2006-949621; Art ID: S02706ST



Scheme 1 *Reagents and conditions*: (a) $(HCHO)_n$, Triton B (40% in MeOH), reflux, 6 h, 96%; (b) $COCl_2$, THF, 0 °C, 3 h, 99%; (c) $NH_2CH_2CH_2NH_2$, 1,4-dioxane–H_2O (2:1), r.t., 3 h, 79%; (d) $BrCH_2CO_2Et$, K_2CO_3, CH_2Cl_2, r.t., 2 h, 87%; (e) TBTU, HOBT, DIEA–DMF, r.t., 12 h, 86–90%; (f) LiOH, EtOH–THF, r.t., 2 h, 89–93%.

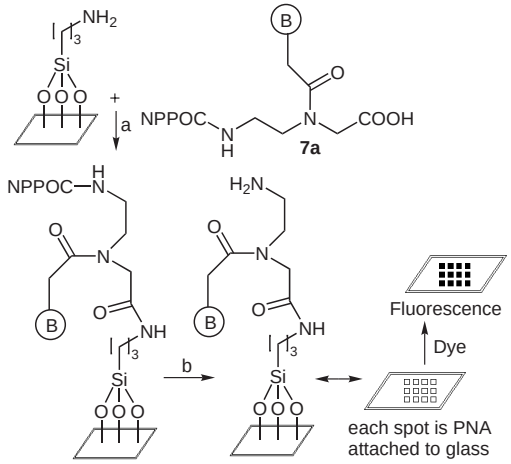


Figure 2 Attachment of NPPOC-protected PNA monomer (T) to the amine-coated glass slide (step a) followed by UV deprotection (step b) and fluorescence detection.

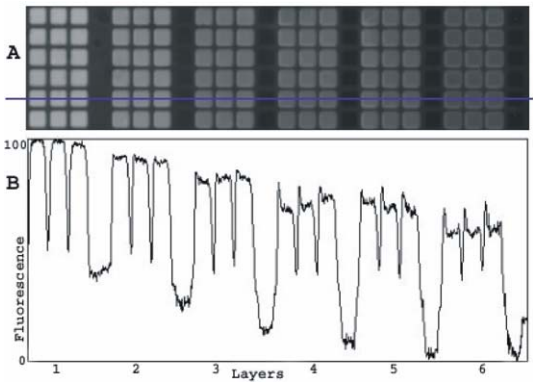


Figure 3 Synthesis (left to right) of mono-, di-, …, and hexa-T using NPPOC-T. A is the scanned slide at 532 nm excitation after the fluorescent dye treatment (pixel size ca. 100 μm); B depicts the intensity of fluorescence at each layer (a stepwise yield of 97%.).

in a flow cell.[7] Specific areas (pixels) of slide were irradiated with DMD (365 nm, 20 mW/cm^2) for different time and then treated with fluorescent dye (BODIPY) followed by scanning on GenPix Scanner (Figure 1). It was found that the optimum NPPOC-T coupling time was 20 minutes, and the photodeprotection time about two minutes in acetonitrile.

Per step coupling efficiency was determined basically as outlined in Figure 2, in which steps a and b were repeated with NPPOC-T by synthesizing one to six layers of T; i.e., after first layer of T was coupled to the amino-glass slide, the pixels corresponding to the second to sixth layers were irradiated, after which the second layer was coupled to the first and so on. Finally all of the T, TT, …, TTTTTT pixels were irradiated to remove NPPOC groups, and the exposed amino groups were coupled to the fluorescent BODIPY dye (Figure 3). In all reactions coupling and capping reactions were carried out according to standard procedures.[18] The fluorescent dye binding and scanning shows about 97% of per step coupling yield.

In conclusion, an efficient method for the synthesis of photolabile 2-(2-nitrophenyl)propyloxy carbonyl (NPPOC)-protected peptide nucleic acids (PNA) for use as building blocks for photolithographic solid-phase PNA synthesis was developed. These derivatives undergo fast light-promoted deprotection with high per step coupling efficiency.

References and Notes

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(15) **Spectroscopic Data for Selected Compounds.**
Compound **4**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.66 (d, *J* = 8.0 Hz, 1 H, ArH), 7.51 (t, *J* = 7.4 Hz, 1 H, ArH), 7.41 (d, *J* = 7.6 Hz, 1 H, ArH), 7.30 (t, *J* = 7.8 Hz, 1 H, ArH), 6.14 (s, 1 H, NH), 4.05–4.25 (m, 2 H, CH_2), 3.59 (m, 1 H, CH), 3.42 (t, *J* = 5.2 Hz, 2 H, CH_2), 3.25 (br s, 1 H, NH_2), 3.10 (t, *J* = 5.6 Hz, 2 H, CH_2), 2.94 (br s, 1 H, NH_2), 1.26 (d, *J* = 6.8 Hz, 3 H, CH_3). MS (API-ES$^+$): *m/z* = 268.1 [M + H$^+$], 290.1 [M + Na$^+$], 306.0 [M + K$^+$].
Compound **5**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.72 (d, *J* = 8.0 Hz, 1 H, ArH), 7.54 (t, *J* = 7.4 Hz, 1 H, ArH), 7.45 (d, *J* = 7.6 Hz, 1 H, ArH), 7.31 (t, *J* = 7.8 Hz, 1 H, ArH), 5.09 (s, 1 H, NH), 4.02–4.21 (m, 2 H, CH_2), 4.09 (q, *J* = 7.2 Hz, 2 H, CH_2), 3.70 (m, 1 H, CH), 3.38 (s, 2 H, CH_2), 3.20 (t, *J* = 5.2 Hz, 2 H, CH_2), 2.76 (t, *J* = 5.6 Hz, 2 H, CH_2), 2.21 (br s, 1 H, NH), 1.27 (d, *J* = 6.8 Hz, 3 H, CH_3), 1.21 (t, *J* = 7.0 Hz, 3 H, CH_3). MS (API-ES$^+$): *m/z* = 354.1 [M + H$^+$], 376.1 [M + Na$^+$], 392.1 [M + K$^+$].
Compound **6a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.52 and 8.50 (2 s, 1 H, NH), 7.67 (d, *J* = 8.0 Hz, 1 H, ArH), 7.52 (t, *J* = 7.4 Hz, 1 H, ArH), 7.43 (d, *J* = 7.6 Hz, 1 H, ArH), 7.30 (t, *J* = 7.8 Hz, 1 H, ArH), 7.01 and 6.98 (2 s, 1 H, CH), 5.63 and 5.05 (2 s, 1 H, NH), 4.38 and 4.32 (2 s, 2 H, CH_2), 4.03–4.20 (m, 2 H, CH_2), 4.08 (q, *J* = 7.2 Hz, 2 H, CH_2), 4.01 (s, 2 H, CH_2), 3.64 (m, 1 H, CH), 3.50 (t, *J* = 5.2 Hz, 2 H, CH_2), 3.32 (t, *J* = 5.6 Hz, 2 H, CH_2), 1.89 (s, 3 H, CH_3), 1.35 (d, *J* = 6.8 Hz, 3 H, CH_3), 1.25 (t, *J* = 7.0 Hz, 3 H, CH_3). MS (API-ES$^+$): *m/z* = 520.1 [M + H$^+$], 542.1 [M + Na$^+$], 558.1 [M + K$^+$].
Compound **7a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.81 and 9.79 (2 br s, 1 H, NH), 7.70 (d, *J* = 8.0 Hz, 1 H, ArH), 7.54 (t, *J* = 7.4 Hz, 1 H, ArH), 7.45 (d, *J* = 7.6 Hz, 1 H, ArH), 7.33 (t, *J* = 7.8 Hz, 1 H, ArH), 7.06 and 7.01 (2 s, 1 H, CH), 5.88 and 5.39 (2 s, 1 H, NH), 4.40 and 4.38 (2 s, 2 H, CH_2), 4.07–4.25 (m, 2 H, CH_2), 4.02 (s, 2 H, CH_2), 3.62 (m, 1 H, CH), 3.49 (t, *J* = 5.2 Hz, 2 H, CH_2), 3.35 (t, *J* = 5.6 Hz, 2 H, CH_2), 1.82 and 1.80 (2 s, 3 H, CH_3), 1.32 (d, *J* = 6.8 Hz, 3 H, CH_3). MS (API-ES$^+$): *m/z* = 492.1 [M + H$^+$], 514.1 [M + Na$^+$], 530.0 [M + K$^+$].

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(17) In a typical procedure to attach photolabile PNA to an amino-glass slide, NPPOC-T was attached to the amino-glass slide mounted in a flow cell by activation of the carboxyl groups of NPPOC-T (14.7 mg, 0.03 mmol) in DMF (0.25 mL). After 20 min, excess NPPOC-T was flushed out with MeCN and specific areas (pixels) were irradiated at 365 nm in MeCN using DMD for varying period of time to remove the photolabile protecting groups at specific locations. The amino groups exposed by this treatment were visualized by being covalently coupled to a BODIPY dye (100 μg) in DMF (0.25 mL) for 10 min followed by scanning on a GenPix Scanner.

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