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A new synthetic protocol for labeled oligonucleotides, using a chemically cleavable universal linker

Shweta Mahajan,[a] S. Patnaik,[a] P. Kumar,[a,*] R. P. Gandhi[b] and K. C. Gupta[a,*]

[a]*Nucleic Acids Research Laboratory, Institute of Genomics and Integrative Biology, Mall Road, Delhi University Campus, Delhi 110 007, India*
[b]*Dr. B.R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi 110 007, India*

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Abstract—A two-step general method for labeling of synthetic oligonucleotides is described. The protocol employs a cleavable universal linker, 5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-benzoyl-2′-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)-uridine phosphoramidite, to effect coupling to polymer-bound oligonucleotide chains. Sequentially, coupling with commercially available phosphoramidite reagent of an appropriate label (Biotin, HEX etc.) in an automated DNA synthesizer is carried out. The labeled oligomers, obtained after cleavage and deprotection reactions, are analyzed on RP-HPLC. A distinctive feature of this protocol is the recovery of free oligomers from their labeled analogs under mild conditions. The oligomers obtained are comparable to the corresponding standard oligonucleotides (HPLC).

1. Introduction

As a result of intensifying concerns over hazards associated with use and disposal of radionuclides, non-radiolabeled biopolymers are being used extensively for a wide variety of applications in modern biology.[1–3] Labeled oligonucleotides and nucleic acids find various applications in detection of amplification products of polymerase chain reactions,[4] solid-phase DNA sequencing,[5] hybridization probes,[6–9] etc. Conventional approaches for synthesis of labeled synthetic oligonucleotides involve introduction of a nucleophilic functional group in the synthetic scheme, followed by reaction with an appropriate electrophilic reporter moiety.[10–13] Also, there exist a few reporter phosphoramidite reagents and engineered supports, which allow one to label either 5′- or 3′-end. Literature records a few methods for permanent attachment of reporter moieties to oligonucleotides,[14–19] albeit, in these protocols, the linker and/or the label cannot be selectively removed to generate free oligonucleotides from their labeled analogs.

The strong affinity between biotin and streptavidin or avidin (association constant 10^{15}/M) has proved to be useful for efficient isolation of biotinylated DNA from complex mixtures.[20,21] Biotinylation of target molecules can be achieved either chemically or enzymatically. A biotin moiety can be introduced into the oligonucleotide chain during solid phase synthesis using its phosphoramidite[22–24] or by performing post-synthetic modification using appropriate biotinylating reagents such as biotin hydrazide and biotin *N*-hydroxysuccinimide ester.[21,25] Biotinylation, if it can be reversed, holds potential as a general approach in molecular biology, since this leads to release of free oligonucleotide from the biotin–streptavidin/avidin complex. As such, a full-length biotinylated oligonucleotide can be isolated from failure sequences, generated during automated solid phase synthesis, using streptavidin/avidin affinity media followed by release of free (unmodified) oligonucleotide for subsequent use. Thus, we reasoned that introduction of a chemically cleavable spacer arm would be advantageous for removal of biotin moiety from a corresponding biotinylated oligonucleotide. Most of the labeling techniques are beset with disadvantage since an oligonucleotide cannot be recovered in its original form, due to absence of a cleavable linkage between oligonucleotide and label. The widespread use of biotin–streptavidin system has led to a need for methods which offer a cleavable bond between the label and nucleic acid.

Keywords: Universal linker; Oligonucleotides; Labeling; Cleavage conditions; Biotin–phosphoramidite; HEX–phosphoramidite; Deprotection.
* Corresponding authors. Tel.: +91 11 27662491; fax: +91 11 27667471; e-mail: kcgupta@igib.res.in

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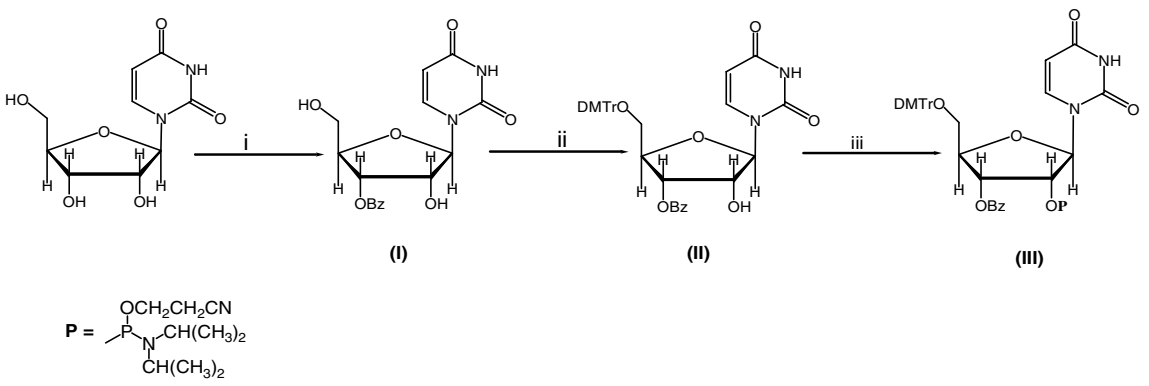
We speculated that a cleavable linker would be useful for manipulation, purification and analysis of chemically synthesized oligonucleotides. An earlier report in this direction relates to incorporation of, for example, a linker containing disulfide linkage between biotin and oligonucleotide, which is then cleaved by a reducing agent such as DTT.[26,27] However, the method admits of a limitation that, during the cleavage of the label from the oligomer, a part of the linker is left on the oligonucleotide, thus returning a modified oligonucleotide. Gildea et al.[28] reported an acid-labile linker for biotinylation of oligonucleotides. Though, this has been successfully used for affinity purification of synthetic DNA, the synthesis of biotinylating reagent required many steps. Rothschild and co-workers reported a photocleavable phosphoramidite for 5′-end labeling, but the formation of thymine–thymine dimer under UV-irradiation is a point of concern and which becomes more significant when long sequences containing more thymidines are involved.[29,30] Towards partial solution of these concerns, Fang and Bergstorm[31] reported two fluoride cleavable biotinylating phosphoramidites for 5′-end labeling, which can be further used for affinity purification of oligonucleotides. However, this approach admits of two main weaknesses: (i) the reagent involves base specific multistep syntheses and (ii) the labeling strategy is limited to single reporter group (biotin). The alternative approach proposed by the same research group also results in 5′-end phosphorylated DNA upon fluoride treatment.[32] In this communication, we report on a new method for 5′-end labeling of oligonucleotides employing a cleavable universal linker and commercially available reporter phosphoramidites, which are not base specific. The highlighting feature of the proposed strategy is that the free oligonucleotide can be recovered back, if required, from its labeled analog under mild conditions.

2. Results and discussion

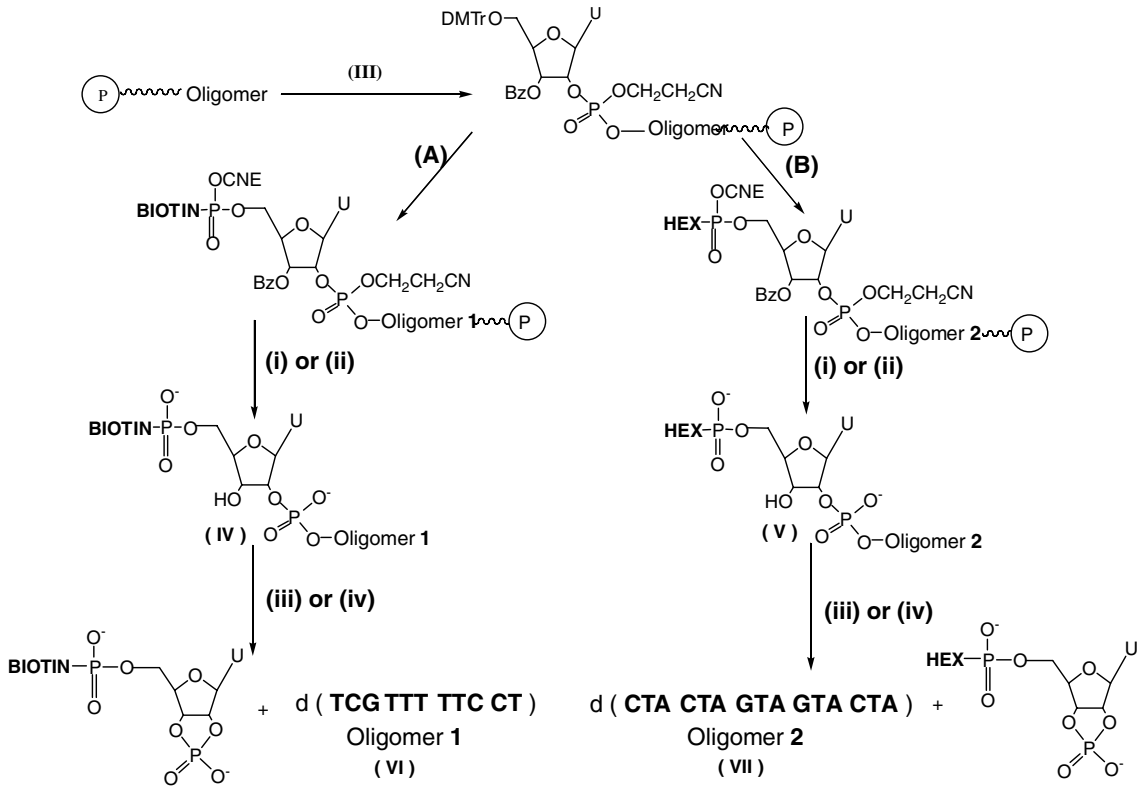
In designing the projected general method for introducing reporter groups in synthetic oligonucleotides through a cleavable linker, we were guided by the following considerations: (a) the method should involve the use of commercially available reporter phosphoramidites in machine-aided synthesis without deviation from the standard protocols, (b) the synthesis of the cleavable linker should be straightforward using commonly available reagents and chemicals in minimal number of steps, (c) the labeled oligomer assembled in the machine should be cleaved from its support along with cleavable linker without loss of reporter group, and (d) the recovery of free oligomer from its corresponding labeled analog should be accomplished rapidly without formation of any side product.

In this investigation, we have employed commercially available reporter phosphoramidites (Biotin and HEX) and designed a cleavable universal linker, 5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-benzoyl-2′-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)-uridine phosphoramidite (**III**) (Scheme 1). The strategy rests on the attachment of linker at 5′-terminal of the growing oligonucleotide chain, followed by coupling with the desired reporter phosphoramidite (Biotin, HEX phosphoramidite, etc.). The oligonucleotide sequence was then deprotected under two different conditions[33] to obtain labeled oligonucleotide in the sequel [(**i**) treatment with anhydrous *tert*-$C_4H_9NH_2$ and methanol (1:1, v/v) for 12 h at rt and lyophilization, followed by deprotection with aq NH_4OH and CH_3NH_2 (40%) (1:1, v/v) for 5 min at 65 °C or (**ii**) treatment with 0.5 M DBU in MeCN for 2 h at rt and lyophilization, followed by deprotection with aq NH_4OH and CH_3NH_2 (40%) (1:1, v/v) for 5 min at 65 °C]. The unmodified oligonucleotides were obtained from their labeled analogs, by subjecting the labeled oligomers to mild condition [(**iii**) 0.2 M NaOH containing 0.5 M NaCl (200 μl) for 30 min at room temperature or (**iv**) 1.0 M spermine containing 1.5 M LiCl (200 μl) for 20 min at 60 °C, Scheme 2], already reported from this laboratory.[34,35]

The projection of the new linker (**III**) rests on our earlier work on universal supports,[34,35] designed for oligonucleotide synthesis, where we reported two sets of fast deprotection conditions for liberation of free oligomers from *cis*-diol group bearing universal linker. It becomes apparent that introduction of this linker at the 5′-end of polymer bound oligomer, followed by coupling with a desired reporter phosphoramidite, would result in a



Scheme 1. Preparation of cleavable universal phosphoramidite linker (**III**). Reagents and conditions: (i) a—Bu_2SnO, reflux, MeOH; b—BzCl, TEA, rt, 10 min; (ii) DMTrCl, py, rt, 24 h; (iii) 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphoramidite, tetrazole, MeCN.



Scheme 2. Methodology for synthesis of labeled oligonucleotides and formation of label free oligonucleotides. (A) biotin–phosphoramidite; (B) HEX–phosphoramidite (**i**) anhydrous *tert*-$C_4H_9NH_2$ and dry CH_3OH (1:1 v/v), 12 h, rt; lyophilization followed by aq $NH_4OH/MeNH_2$ (40%) (1:1 v/v), 5 min, 65 °C; (**ii**) 0.5 M DBU in MeCN, 2 h, rt; lyophilization followed by aq $NH_4OH/MeNH_2$ (40%) (1:1 v/v), 5 min, 65 °C; (**iii**) 0.2 M NaOH containing 0.5 M NaCl for 30 min, rt; (**iv**) 1.0 M spermine containing 1.5 M LiCl for 20 min, 60 °C.

labeled oligomer with cleavable property. The two-step process would permit use of a reporter group of choice and also obviate the need to prepare a large number of phosphoramidite reagents of reporter groups bearing cleavable linker or reporter group bearing nucleosides. The synthesis of cleavable linker (**III**), its incorporation, and subsequent labeling with desired reporter groups during solid phase synthesis are illustrated in Schemes 1 and 2. The linker (**III**) was prepared from (**I**)[36] through reaction with DMTrCl at room temperature to give (**II**) (82% yield, characterized by IR, ^{1}H NMR, elemental analysis, and low-resolution MS), which was phosphitylated using 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphoramidite to yield (**III**) in 89% yield (Scheme 1).

In order to examine the effect of fast deprotection conditions on cleavage of labeled oligomers, a model experiment was designed in which the last coupling was performed with universal linker (**III**) on a pre-synthesized support-bound oligomer, d(TTT TTT TTT T), followed by coupling with reporter phosphoramidite (B = HEX) with an extended coupling time (5 min). The labeled oligomer was then subjected to deprotection condition [(**i**) or (**ii**)], followed by desalting and analysis on RP-HPLC. The elution profile of purified labeled analog obtained after subjecting it to cleavage condition (**i**) is depicted in Figure 1a. The identity of the eluent was established by co-injecting it with standard d(TTT TTT TTT T), as shown in Figure 1b. Figure 1c shows elution profile of purified labeled d(TTT TTT TTT T), obtained after treatment under cleavage condition (**ii**).

To demonstrate general utility of the projected strategy, oligonucleotide sequences, d(TCG TTT TTC CT) (oligomer **1**) and d(CTA CTA GTA GTA CTA) (oligomer **2**), were assembled on standard dT and dA supports, respectively, at 0.2 μmol scale using labile nucleoside phosphoramidites (dA^{pac}, dC^{ac}, and dG^{pac}). In the present study, *N*-acetyl-DMTrdC-phosphoramidite was employed to avoid any modification occurring at the deprotection stage with CH_3NH_2.[33] Labeling of oligomers (**1** and **2**) was achieved in two steps in an automated DNA synthesizer itself[37] (as described above). Subsequently, the polymer-bound oligomers, viz., Biotin–U–d(TCG TTT TTC CT) and HEX–U–d(CTA CTA GTA GTA CTA), were subjected to deprotection condition (**ii**) as mentioned above to get rid of the protecting groups from nucleic bases and internucleotidic phosphates while keeping the universal linker intact. After usual workup and desalting, the labeled oligomers were concentrated in a Speed Vac followed by HPLC purification. The purified products were redissolved in water, divided into three portions, and lyophilized.

A portion of the labeled oligomer **1** (**IV**) was dissolved in 0.1 M NH_4OAc buffer, and analyzed on RP-HPLC. The elution profile of the purified labeled oligomer **1** (**IV**) is

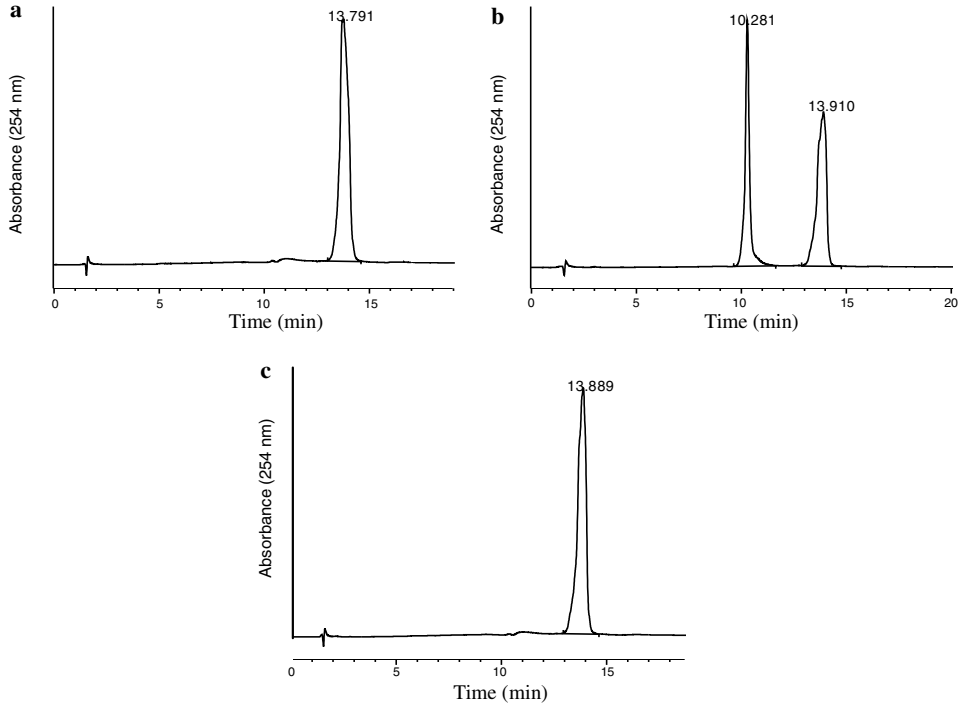


Figure 1. RP-HPLC profiles of (a) purified oligomer, HEX–U–d(TTT TTT TTT T), obtained after cleavage [under condition (**i**)], (b) co-injection of purified oligomer, HEX–U–d(TTT TTT TTT T) and standard d(TTT TTT TTT T), and (c) purified oligomer, HEX–U–d(TTT TTT TTT T), obtained after cleavage [under condition (**ii**)].

shown in Figure 2a. The same oligomer **1** (**IV**) was co-injected with the corresponding standard oligomer d(TCG TTT TTC CT) and the elution pattern is shown in Figure 2b. The second portion of oligomer **1** (**IV**) was subjected to deprotection condition (**iii**) to cleave the universal linker along with biotin moiety.[34] The cleavage of the universal linker from (**IV**) occurs presumably via a cyclic phosphate (cf. Ref. 35). The resulting solution, after neutralization with acetic acid, was concentrated in a Speed Vac, redissolved in NH_4OAc buffer and analyzed on RP-HPLC (Fig. 2c). The third portion was subjected to mild condition (**iv**) followed by usual work-up, as described above. The residue was redissolved in buffer and analyzed on RP-HPLC. The elution profile of the oligomer **1** (**VI**) obtained after above treatment is shown in Figure 2d. In both cases, the free oligomer **1** (**VI**) eluted with a lower retention time as compared to labeled oligomer **1** (**IV**). The identity of the released free oligonucleotides (in both the cases) was further confirmed by co-injecting them with the corresponding standard oligomer, d(TCG TTT TTC CT). Figure 2e shows elution pattern of a co-injection of oligomer **1** (**VI**), obtained after subjecting oligomer **1** (**IV**) to cleavage condition [(**iii**), Scheme 2] with the corresponding standard oligomer on RP-HPLC.

Likewise, the purified labeled oligomer **2** (**V**) was dissolved in water, divided into three portions, and lyophilized. A portion of oligomer **2** (**V**) was directly redissolved in NH_4OAc buffer and analyzed on RP-HPLC, whereas the remaining two portions of oligomer **2** (**V**) were subjected to different deprotection conditions[34,35] and then analyzed on RP-HPLC. Figures 3a and b sketch elution profiles of oligomer **2** (**V**) and a co-injection of oligomer (**V**) with (**VII**), respectively. Figures 3c and d depict crude oligomer **2** (**VII**) after cleavage under conditions (**iii**) and (**iv**), respectively. Figure 3(e) shows a co-injection of crude oligomer **2** (**VII**), obtained after treatment of oligomer **2** (**V**) to cleavage condition [(**iv**), Scheme 2] with standard oligomer, d(CTA CTA GTA GTA CTA), on RP-HPLC.

In order to demonstrate the practical applicability of the cleavable linker (**III**) and usefulness of affinity purification of biotinylated probes, the purified biotinylated oligomer **1** (**IV**) was incubated with magnetic Dynabeads M-280 streptavidin in binding buffer. Subsequently, the beads were subjected to cleavage condition (**iii**) (Scheme 2). After usual work-up, the cleaved oligomer **1** (**VI**) was analyzed on RP-HPLC. The oligomer **1** (**VI**), as anticipated, eluted with the standard oligomer when the co-injection was analyzed on RP-HPLC, as also shown in Figure 4.

3. Conclusion

A new universal linker based on uridine nucleoside has been synthesized and used to incorporate cleavable property in labeled oligonucleotides. It has been demonstrated that the linker phosphoramidite can be coupled to the 5′-end of synthetic oligonucleotides efficiently in an automated DNA synthesizer with subsequent coupling with the commercially

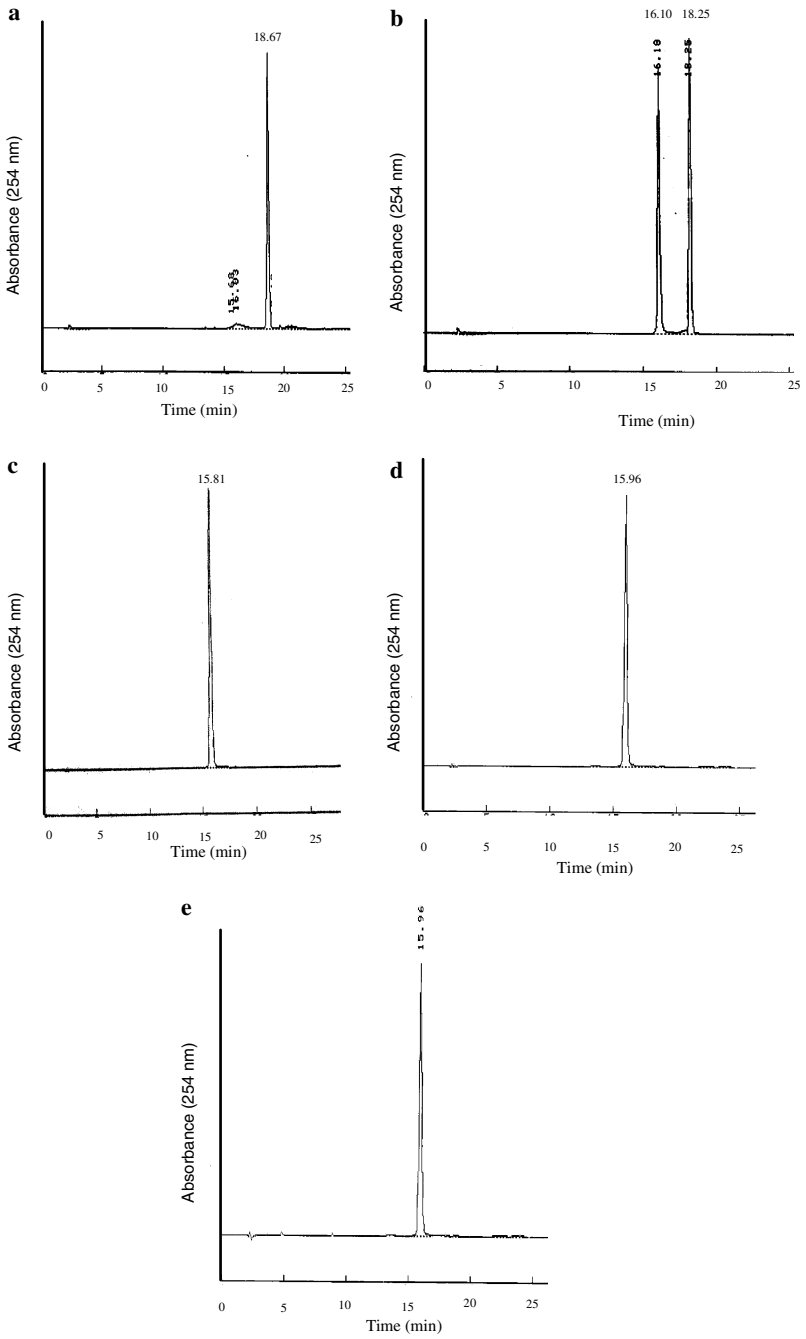


Figure 2. RP-HPLC profiles of (a) purified oligomer, Biotin–U–d(TCG TTT TTC CT) (**IV**), obtained after cleavage [under condition (**i**)], (b) co-injection of Biotin–U–d(TCG TTT TTC CT) (**IV**) and standard d(TCG TTT TTC CT), (c) d(TCG TTT TTC CT) (**VI**) obtained after treatment of (**IV**) with (**iii**), (d) d(TCG TTT TTC CT) (**VI**) obtained after treatment of (**IV**) with (**iv**), and (e) co-injection of d(TCG TTT TTC CT) (**VI**), obtained after treatment of (**IV**) with (**iii**), with standard d(TCG TTT TTC CT). 'U' denotes chemically cleavable linker (**III**).

available desired labeled phosphoramidite. The linker between the label and the oligonucleotide is sufficiently stable during post-synthesis work-up, however, can readily be detached from the labeled oligonucleotides under mild condition, (**iii**) or (**iv**). The simplicity of the method, comprising usage of commercially available reporter phosphoramidites and straightforward synthesis of cleavable linker arm from commonly available chemical reagents in a minimal number of steps, is reflective of new possibilities of manipulations in the DNA and RNA chemistry.

4. Experimental

4.1. 3′-*O*-Benzoyluridine (I)

This was prepared according to the literature procedure[36] and characterized by ^{1}H NMR and IR spectroscopy.

4.2. Preparation of 5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-benzoyluridine (II)

3′(2′)-*O*-Benzoyluridine (1.0 g, 2.87 mmol) was dried by co-evaporation with anhydrous pyridine and dis-

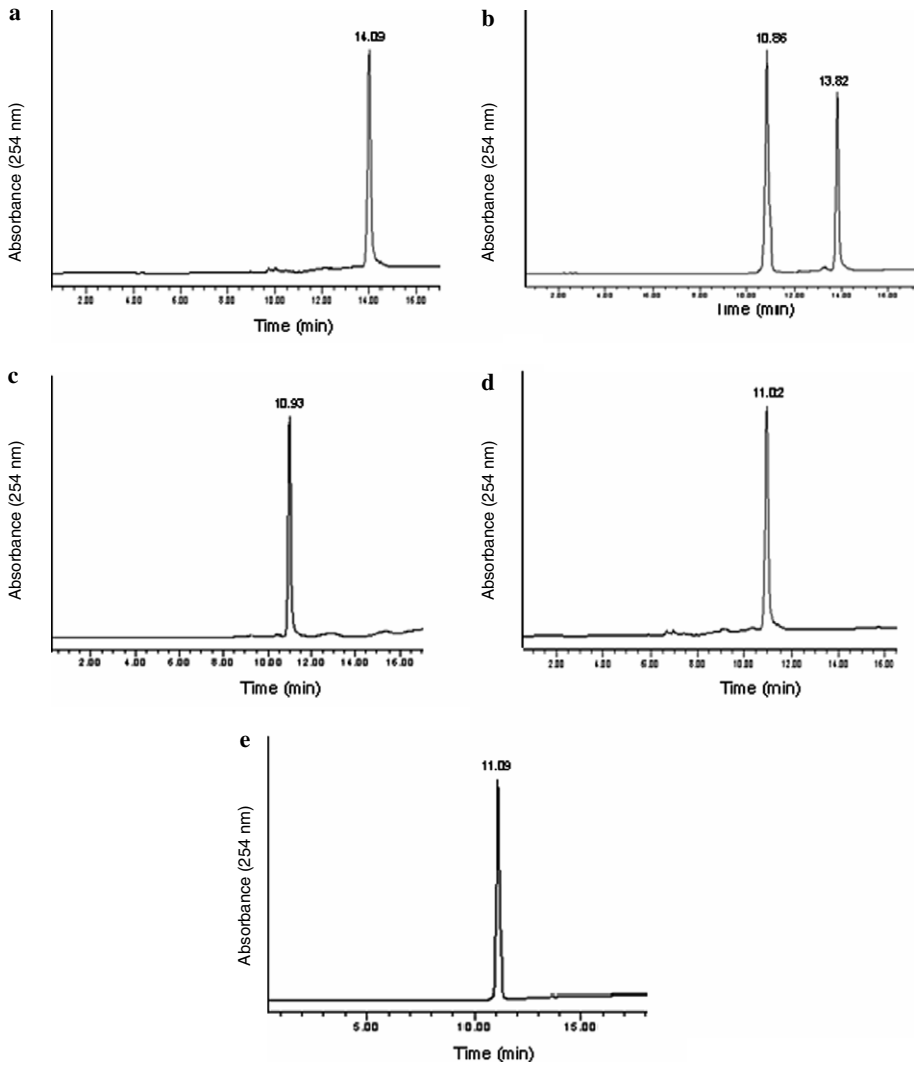


Figure 3. RP-HPLC profiles of (a) purified oligomer, HEX–U–d(CTA CTA GTA GTA CTA) (**V**), obtained after cleavage [under condition (**ii**)], (b) co-injection of HEX–U–d(CTA CTA GTA GTA CTA) (**V**) with standard d(CTA CTA GTA GTA CTA), (c) d(CTA CTA GTA GTA CTA) (**VII**) obtained after treatment of (**V**) with (**iii**), (d) d(CTA CTA GTA GTA CTA) (**VII**) obtained after treatment of (**V**) with (**iv**), and (e) co-injection of d(CTA CTA GTA GTA CTA) (**VII**), obtained after treatment of (**V**) with (**iv**), with standard d(CTA CTA GTA GTA CTA). 'U' denotes chemically cleavable linker (**III**).

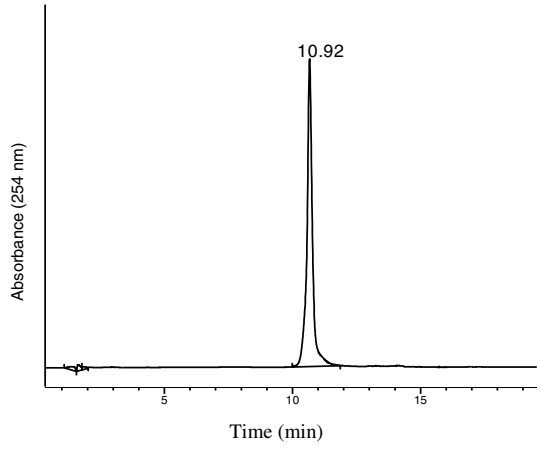


Figure 4. RP-HPLC profile of d(TCG TTT TTC CT) (**VI**) obtained after cleavage from Dynabeads M-280 Streptavidin under cleavage condition (**iii**).

solved in dry pyridine (10 ml). 4,4′-Dimethoxytrityl chloride (DMTrCl) (1.1 g, 3.2 mmol) was added and the reaction mixture was stirred at room temperature for 6 h. Methanol (1 ml) was then added to quench the reaction and the solution was concentrated to an oil, which was re-dissolved in EtOAc (75 ml) and washed with 5% aqueous $NaHCO_3$ solution (3× 25 ml) and later with saturated NaCl solution (2× 25 ml). The organic phase was collected and concentrated on a rotary evaporator to a syrupy material. It was redissolved in 1,2-dichloroethane (EDC) containing triethylamine (TEA) (1%, v/v) and purified by silica gel column chromatography using a stepwise gradient of CH_3OH (0.5–2%) in EDC containing TEA (1%, v/v). The fractions containing the desired material were pooled together and concentrated to obtain the title compound as a light brown solid in 82% (1.49 g) yield.

R_f : 0.51 (DCM/CH_3OH 9:1). IR (thin film) ν (cm^{-1}) = 1033, 1255, 1682, 1702, 3049, 3502. ^1H NMR ($CDCl_3$) δ (ppm) : 3.16 (d, 2H, 5′-CH_2), 3.79 (s, 6H, 2× OCH_3), 4.25 (m, 1H, 4′-CH), 4.45 (br, 1H, 2′-CH), 4.95 (br, 1H, 3′-CH), 5.95 (m, 1H, 1′-CH), 6.70–7.60 (m, 19H, Ar-H, 5-CH), 8.05 (d, 1H, 6-CH). MALDI-TOF = 673.34 ($M+Na^+$). Anal. Calcd for $C_{37}H_{34}N_2O_9$: C, 68.31; H, 5.23; N, 4.31. Found : C, 66.21; H, 5.34; N, 4.17.

4.3. 5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-benzoyl-2′-*O*-2-cyanoethyl-*N*,*N*′-diisopropyl- uridine phosphoramidite (III)

A round-bottomed flask containing 5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-benzoyluridine (500 mg, 0.76 mmol) was flushed with argon and charged with anhydrous CH_3CN (10 ml). 2-Cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropyl phosphoramidite (457.5 μl, 1.52 mmol) was added dropwise via a syringe. A solution of 1*H*-tetrazole (53.2 mg, 0.76 mmol), dissolved in dry CH_3CN (2 ml), was added dropwise over a period of 5 min. After stirring at room temperature for 2 h (completion monitored on TLC), the reaction was quenched by adding methanol (1 ml). The reaction mixture was concentrated under reduced pressure and the residue taken up in EtOAc (75 ml) containing TEA (0.5%, v/v). The organic phase was washed with saturated NaCl solution (2× 25 ml) and dried over anhydrous Na_2SO_4. After filtration, the solvent was removed and the residue redissolved in EDC containing TEA (1%, v/v) and purified on silica gel column. The fractions containing the desired material were pooled together and concentrated under reduced pressure to obtain the compound (**III**) in 89% (583 mg) yield. R_f: 0.68 (EtOAc/DCM/TEA 4.5:4.5:1).

4.4. *N*-1-(4,4′-Dimethoxytrityl)biotinyl-aminohexanol phosphoramidite (A)

This was prepared according to the protocol published earlier from this laboratory.[16]

4.5. Oligonucleotide synthesis, deprotection, and purification

The oligonucleotides were synthesized at 0.2 μmol scale on a Pharmacia LKB Gene assembler Plus using standard β-cyanoethyl phosphoramidite chemistry, with conventional (dA^{bz}, dC^{ac}, and dG^{ibu}) and labile (dA^{pac}, dC^{ac}, and dG^{pac}) nucleoside phosphoramidites. For tethering, biotin at the 5′-terminal of the synthesized oligomer, the universal linker phosphoramidite (**III**) (0.2 M) was dissolved in absolute CH_3CN and coupled to 5′-terminal hydroxyl groups of the oligomer sequence with extended coupling time (5 min) followed by coupling of biotin–phosphoramidite (**A**) again with extended coupling reaction time (5 min). Similarly, HEX–phosphoramidite (**B**) was attached to the oligomer sequence bound to polymer support with an extended coupling time (5 min) after coupling of the universal linker (**III**). Subsequently, the support-bound oligomers were subjected to treatment with either (**i**) anhydrous *tert*-$C_4H_9NH_2$ and methanol (1:1, v/v) for 12 h at rt and lyophilization, followed by deprotection with aq NH_4OH and CH_3NH_2 (40%) (1:1, v/v) for 5 min at 65 °C or (**ii**) 0.5 M DBU in MeCN for 2 h at rt and lyophilization, followed by deprotection with aq NH_4OH and CH_3NH_2 (40%) (1:1, v/v) for 5 min at 65 °C. These treatments resulted in cleavage of oligonucleotide chains from the support as well as removal of the protecting groups from nucleic bases and internucleotidic phosphates.

The oligomers were concentrated and subjected to desalting on a RP-18 silica gel column. Elution was realized with 30% acetonitrile in water and concentrated in vacuo. After purification, these were redissolved in water, divided into three portions, and lyophilized. One portion of the desalted oligomers was dissolved in NH_4OAc buffer, pH 7.1, and analyzed on RP-HPLC. Other portions of oligomers were subjected to following treatments in order to obtain free oligomers:

(a) Oligomer with Biotin label (**IV**) was dissolved in a solution (200 μl) of 0.2 M NaOH containing 0.5 M NaCl and kept at rt for 30 min. The solution was neutralized with acetic acid (20 μl) and concentrated in a Speed Vac. The material was dissolved in water, desalted, concentrated, redissolved in NH_4Ac buffer, and analyzed on RP-HPLC. A similar treatment of HEX-labeled oligomer (**V**) resulted in a label free oligomer (**VII**).
(b) Alternatively, the oligomer with Biotin label (**IV**) was treated with a solution (200 μl) of spermine (1.0 M) and LiCl (1.5 M) at 60 °C for 20 min. After usual work-up, the fully deprotected oligomer (**VI**) was analyzed on RP-HPLC. After a similar treatment, the HEX-labeled oligomer (**V**) was analyzed on RP-HPLC.

The above fully deprotected oligomers (**VI** and **VII**) were compared with corresponding oligomers, which were synthesized on standard supports and deprotected following standard protocol.[15]

4.6. Affinity purification of biotinylated probe

Oligomer sequence, Biotin–U–d(TCG TTT TTC CT) (oligomer **1**) (**IV**), (0.4 O.D. A_{254}), obtained after deprotection with aq NH_4OH and CH_3NH_2, was dissolved in a binding buffer, 10 mM Tris–HCl, pH 7.5, containing 1 mM EDTA and 1.0 M NaCl. It was then mixed with magnetic Dynabeads M-280 Streptavidin (10 mg), pre-washed with PBS (0.1 M phosphate buffer containing 0.5 M NaCl) buffer, pH 7.2 (2× 1.0 ml). After incubation for 1 h at rt, the Eppendorf tube containing the above suspension was kept on a magnetic particle concentrator. The supernatant liquid was removed and the beads were repeatedly washed with binding buffer (3× 1 ml). The beads were treated with a solution of 0.2 N NaOH containing 0.5 M NaCl for 30 min at rt. The supernatant liquid was removed, neutralized, concentrated, and worked up as described above. The cleaved oligomer (**VI**) was then analyzed on RP-HPLC.

Acknowledgments

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