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Photocaging Strategy for Functionalisation of Oligonucleotides and Its Applications for Oligonucleotide Labelling and Cyclisation

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Abstract: We have used a photocaging strategy to develop novel phosphoramidites and expand the repertoire of protecting groups for modification of oligonucleotides by solid-phase synthesis. We synthesised five photolabile phosphoramidites and four new photolabile controlled pore glasses (CPGs). By using these photolabile phosphoramidites and CPGs, modified oligodeoxynucleotides (ODNs) with phosphate, amine, acid, thiol and carbonyl moieties at 5′ and/or 3′ ends were readily synthesised. To the best of our knowledge, this is the first report of introducing a carbonyl at the 5′ end and thiol groups at both ends of ODNs with photolabile modifiers. Terminal labelling was also easily realised in solution or by on-column solid-phase synthesis. By using the photolabile amine modifier and the photolabile acid CPG, cyclisation of an oligodeoxynucleotide was achieved with good yields. This study provides an alternative way to introduce functional groups into oligonucleotides and expand the scope of oligonucleotide bio-orthogonal labelling.

Keywords: caged compounds · oligonucleotide modifiers · oligonucleotides · photoactivation · photolabile phosphoramidites

Introduction

Oligonucleotides altered by the attachment of functional groups are very useful for oligonucleotide labelling and conjugation. These modified oligonucleotides display a variety of applications in many fields, including antisense drug development, DNA microarray[1] and sequence analysis,[2] nanotechnology[3] and in vivo imaging.[4] Many functional groups such as amine, acid and thiol groups have been incorporated into oligonucleotides by using corresponding modifiers, and some of them are commercially available and widely used.[5–22] Despite these successful methods for oligonucleotide modification, there is still room to improve and expand the development of protected functional groups for oligonucleotide solid-phase synthesis, especially in the following circumstances: 1) When an oligonucleotide is labelled with multiple moieties through consecutive deprotection of amine protecting groups and formation of amide bonds, a photocaging strategy may become a good choice in addition to 4-methoxytriphenylmethyl (MMTr) and fluorenylmethyloxycarbonyl (Fmoc)/trifluoactate chemistry. 2) Conjugation of oligonucleotides with larger molecules, such as peptides, is of significant interest due to the properties and potential applications of such conjugates.[23–25] The current widely used conjugation approaches of deprotected oligonucleotides with peptides suffer from moderate yields and laborious purification,[26,27] especially with positively charged peptides. The solution-phase conjugation of protected oligonucleotides has advantages over the above methods.[28] 3) Oligonucleotides with attachments of both amine and acid moieties are usually achieved in multiple steps. In addition, cyclisation of these deprotected oligonucleotides through amide bond formation always requires large excesses of reagents and long reaction times, produces low product yields and results in significant amounts of non-specific covalent modification.[29] Photolabile moieties, also known as caged compounds, have shown significance and wide applications in chemistry and biology.[30–35] Photocaged phosphate,[36] amino[28,37,38] and carboxyl groups[39,40] for modified oligonucleotides were previously achieved by Greenberg et al. They showed promising applications for bioconjugation with biotin, peptide and other small molecules. However, a long irradiation time may be needed for photolysis of the 3, 4-dimethoxy-2-nitrobenzyl (DMNB) moiety due to the slow photocleavage kinetics.[41,42] Here we report the synthesis of modified oligodeoxynucleotides with photolabile functional groups that include phosphate, amine, acid, thiol and carbonyl moieties at 5′ and/or 3′ ends to expand the repertoire for the functionality of oligodeoxynucleotides. Terminal labelling with fluorophores at 5′ or 3′ ends was successfully achieved, respectively. Additionally, a circular oligodeoxynucleotide was synthesised after photodeprotection of photolabile amine and acid groups at 5′ and 3′ ends, respectively, followed by coupling of the protected oligodeoxynucleotide in DMF and deprotection of the cyclised product by ammonium hydroxide.

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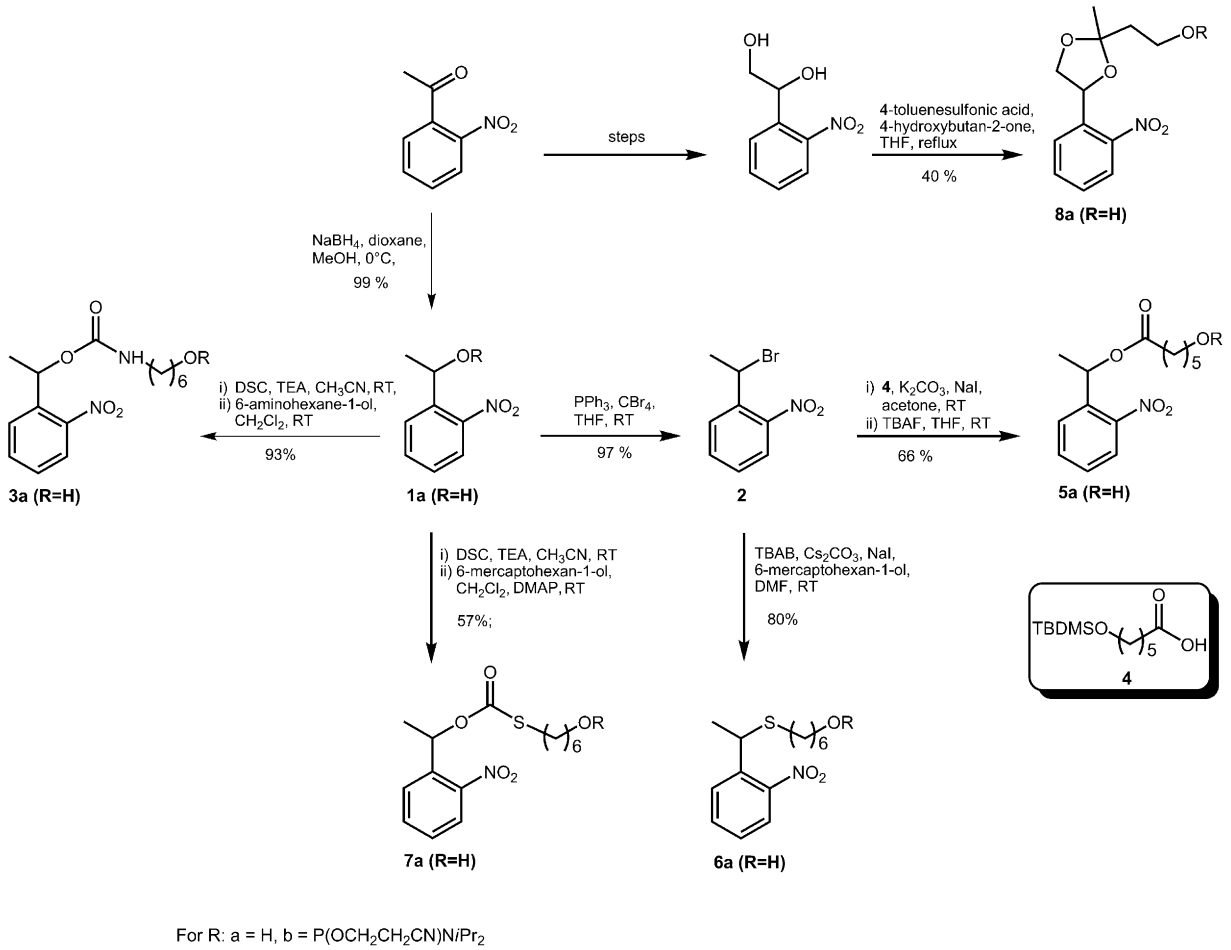
Results and Discussion

Functionalisation of oligodeoxynucleotides with caged 5′-phosphate, amine, acid, thiol and carbonyl moieties: Photolabile moieties are widely used for temporarily controlled protection of functional groups in many applications. To be useful for oligonucleotide modification, photolabile moieties should be stable during DNA synthesis cycles. The 2-nitrophenyl ethyl moiety is capable of withstanding the conditions of DNA solid-phase synthesis and is a good candidate for protecting functional groups on olignucleotides because it has reasonable photocleavage efficiency and faster cleavage kinetics than the 3,4-dimethoxy-2-nitrobenzyl moiety.[43] To make direct attachments of functional moieties to the 5′ end of oligonucleotides by solid-phase synthesis, phosphoramidites are usually used. Photolabile phosphoramidite monomers with phosphate, amine, acid, thiol and carbonyl moieties at the 5′ end were synthesised by using photolabile groups as temporary protecting groups, followed by phosphoramidition as shown in Scheme 1.

To introduce a phosphate at the 5′ end of an oligodeoxynucleotide, 1-(2-nitrophenyl) ethyl phosphoramidite (**1b**) (Scheme 1) was synthesised according to a previous report[44] and then incorporated as the last coupling *X* of an oligodeoxynucleotide sequence (5′ *X* TTT TTC TCT CTC TCT G-3′, **ODN 1**, Table 1). After deprotection and cleavage from CPG, the oligodeoxynucleotide with the hydrophobic caging group has a longer retention time during purification by RP-HPLC. HPLC traces of **ODN 1** show two peaks

Table 1. Oligonucleotide sequences and modifications used in this study.

5′ *X* TTT TTC TCT CTC TCT G 3′		
ODN 1	X = phosphate	**1b**
ODN 2	X = amine	**3b**
ODN 3	X = carboxylic acid	**5b**
ODN 4	X = thiol (via sulfoether)	**6b**
ODN 5	X = thiol (via carbonothioate)	**7b**
ODN 6	X = ketone	**8b**
5′ TTT TTC TCT CTC TCT *Y* 3′		
ODN 7	Y = phosphate	**11b**
ODN 8	Y = amine	**12b**
ODN 9	Y = carboxylic acid	**13**
ODN 10	Y = thiol (via carbonothioate)	**14b**
5′ *X* TTT TTC TCT CTC TCT *Y* 3′		
ODN 11	X = amino Y = carboxylic acid	



Scheme 1. Syntheses of photolabile protecting groups and photolabile phosphoramidites for 5′-end modification of oligonucleotides. Reagents and conditions for **1a**/**3a**/**5a**/**6a**/**7a**/**8a** to **1b**/**3b**/**5b**/**6b**/**7b**/**8a**: $P(OCH_2CH_2CN)NiPr_2Cl$, TEA, dichloromethane, 0 °C, 54 to 74 %. DMAP = 4-dimethylaminopyridine, DSC = *N*,*N*′-disuccinimidyl carbonate, TBAB = tetrabutylammonium bromide, TBAF = tetrabutylammonium fluoride, TEA = triethylamine.

close to each other with similar peak intensity (see Figure S1 in the Supporting Information). Further MS results indicate that these two fractions have the same molecular weight. After irradiation of both oligodeoxynucleotide fractions with UV light for 10 min (360 nm, 11 mW cm^{-2}) to remove the photolabile chiral 1-(2-nitrophenyl) ethyl moiety, followed by HPLC separation, both samples showed the same retention time and same molecular weight. The separation of the two oligodeoxynucleotide isomers on the RP-HPLC column may be due to the short distance between the chiral 1-(2-nitrophenyl) ethyl moiety and the helix of DNA oligodeoxynucleotide, as evidenced by two peaks at 147.7 and 147.3 ppm in the P^{31} NMR spectra of **1b**. The chiral effect of the 1-(2-nitrophenyl) ethyl moiety disappeared with elongation of the distance between the chiral moiety and an oligodeoxynucleotide (caged **ODN 2**), and only a single peak was observed on HPLC traces for caged **ODN 2**.

In the case of the caged amine phosphoramidite **3b**, we first synthesised **3a** with the reaction of 1-(2-nitrophenyl) ethanol and DSC, followed by the addition of hydroxyhexyl amine by using a similar synthetic strategy as literature reports.[45] After phosphoramidition of **3a**, the obtained **3b** was incorporated into the oligodeoxynucleotide sequence (**ODN 2**, Table 1) with the same coupling conditions as normal phosphoramidites. After ammonium hydroxide hydrolysis, HPLC traces show a main single peak, which was proved to be the expected caged amine modified oligodeoxynucleotide by MS (Table 2). Upon photolysis, the caging group was removed and a new peak with the shorter retention time than caged **ODN 2** on HPLC traces appeared. MS confirmed that the new fraction was the uncaged amine-modified oligodeoxynucleotide **ODN 2**, as shown in Table 2.

Table 2. ESI-TOF/MALDI-TOF results and HPLC retention times for the modified oligodeoxynucleotides.

		Expected MW [Da]	Measured MW [Da]	Retention time [min]
5′-end caged	**ODN 1**	4981	4984	21.98
	ODN 2	5124	5127	24.62
	ODN 3	–	–	–
	ODN 4	5097	5100	24.12
	ODN 5	–	–	–
	ODN 6	5067	5071	21.85
5′-end irradiated	**ODN 1**	4832	4834	10.14
	ODN 2	4931	4934	12.39
	ODN 3	4946	4949	9.59
	ODN 4	4949	5099[a]	17.07
	ODN 5	4948	4949	15.28
3′-end irradiated	**ODN 6**	4902	4907	11.75
	ODN 7	4503	4504	10.28
	ODN 8	4602	4607	11.53
	ODN 9	4617	4619	9.79
	ODN 10	4619	4617	12.04
ODN 2-TAMRA		5343	5345	21.45
ODN 3-pyrene		5159	5163	28.25
ODN 8-pyrene		4872	4874	28.06
ODN 11 linear		4796	4799	14.29[b]
ODN 11 vircular		4778	4783	14.67[b]

[a] Photoirradiated immediate was obtained. [b] These results were performed on analysis HPLC column.

To synthesise caged acid and thiol phosphoramidites (**5b**, **6b**), 6-(*tert*-butyldimethylsilyloxy) hexanoic acid and 6-mercapto-1-hexanol were used to react with 1-(1-bromoethyl)-2-nitrobenzene by substitution of the bromo group of **2** to obtain **5a** and **6a** as shown in Scheme 1. After phosphoramidition of **5a** and **6a**, **5b** and **6b** were obtained and attached to the 5′ end of oligodeoxynucleotides on solid phase according to the similar procedure for the synthesis of the caged amino-modified oligodeoxynucleotide. To obtain the acid-functionalised oligodeoxynucleotide **ODN 3**, photolysis is needed to release the acid group. The solid CPG with the modified oligodeoxynucleotide of caged **ODN 3** suspended in acetonitrile/water (4:1) was first irradiated by UV lamp. Then the irradiated CPG can be directly used for further conjugation on the solid phase with other molecules, such as fluorophores and peptides. To further characterise **ODN 3**, we hydrolysed the irradiated CPG of **ODN 3** with ammonium hydroxide. The obtained **ODN 3** was separated by HPLC and confirmed by MS (Table 2). Oligodeoxynucleotides with an acid group at the 5′ end could also be used for coupling with amine-containing base-sensitive molecules or proteins. Direct uncaging of a protected carboxylic acid on oligodeoxynucleotides has advantages to previous efforts of acid functionalisation of oligodeoxynucleotides through two-step coupling by using an amine phosphoramidite and succinic anhydride.[29]

Using standard DNA synthesis cycles for the caged thiol modified ODN with **6b** for the last coupling, we failed to obtain a main peak for the caged thiol-modified oligodeoxynucleotide **ODN 4** on HPLC separation traces. Previous literature reported that the oxidation step with iodine caused the removal of the S-trityl group and dimerisation of free thiol-oligodeoxynucleotides.[46] However reduction of the obtained oligodeoxynucleotide with dithiothrietol (DTT) was ineffective and could not restore the activity of free thiol groups. MS results of the separated fractions from HPLC showed a mix of molecule weights of 5100 Da, which is the expected caged **ODN 4**, and 5132 Da, which is in agreement with the oxidation of sulfoether to sulfone by I_2/pyridine/H_2O/THF. A shorter oxidation time or replacement of the oxidant mixture by carbon tetrachloride/triethylamine/*N*-methylimidazole/H_2O lowered the possibility of oxidation of sulfoether. Photoirradiation of purified caged **ODN 4** produced a new single peak on HPLC trace with shorter retention time (see Figure S2 in the Supporting Information). However, MS results indicated the MW of the new fraction was the same as the MW of caged **ODN 4**. Further treatment of this new irradiated product with DTT failed to recover thiol-modified oligodeoxynucleotide **ODN 4**. According to the literature report,[47] the free thiol moiety can react with a nitroso group to form an N–S bond, which may also be occurring with this oligodeoxynucleotide This observation is different from previous reports of the caged cysteine moiety in proteins that showed full recovery of bio-

logical activities after photouncaging.[48] To overcome the problem with our thiol modification, **7b** was synthesised after phosphoramidition of **7a** (Scheme 1) for the synthesis of carbonothioate protected oligodeoxynucleotide **ODN 5**. Direct treatment by ammonium hydroxide generated a main peak on HPLC trace with a molecular weight of 5002 Da by MALDI TOF, which is probably the oligodeoxynucleotide with thiol-acrylonitrile adduct (calcd MW 5001 = 4948 (**ODN 4**) + 53 (CH_2=CHCN)). With the addition of DTT to ammonium hydroxide during deprotection, the expected thiol-modified oligodeoxynucleotide (MW = 4949 Da) was obtained (Table 2 and Figure S4 in the Supporting Information).

Aldehyde and ketone functional groups are occasionally incorporated into oligonucleotides for use in conjugation to specific moieties such as peptides containing nucleophilic groups (aminooxy, aminothiol, or hydrazine, etc.).[49,50] These conjugation reactions are efficient but suffer from poor stability of aldehyde or ketone on oligonucleotides due to a known propensity to react with purine bases.[17] Previously, the aldehyde moiety has been incorporated into oligonucleotides through post-transformation. Usually acetate-protected diol or protected serine residue is first coupled to an oligonucleotide, followed by careful oxidation after deprotection. Here, we applied a photolabile 1-(2-nitrophenyl) ethane-1,2-diol as the protecting group for 7-hydroxybutan-2-one and synthesised phosphoramidite **8b** (Scheme 1). Caged **ODN 6** with protected ketone at the 5′ end was then synthesised with **8b** under standard DNA "DMT-ON" solid-phase synthesis cycles. After ammonium hydroxide deprotection and HPLC purification, caged **ODN 6** was confirmed by ESI (Table 2 and Figure S4 in the Supporting Information). Upon photolysis, a new oligodeoxynucleotide product with shorter retention time was collected and was confirmed to be ketone-modified oligodeoxynucleotide **ODN 6** by MALDI TOF (Table 2 and Figure S4 in the Supporting Information).

To study the photocleavage kinetics of aqueous caged oligodeoxynucleotide solutions, caged phosphate **ODN 1** and amine modified **ODN 2** (20 μM) were irradiated with light (365 nm, 11 m Wcm^{-2}) and the formation of resulting uncaged modified oligodeoxynucleotides was monitored by HPLC. As seen in Figure 1a, photodeprotection of caged **ODN 1** and **ODN 2** in aqueous solutions was fast and the uncaging efficiencies were over 95% in 15 min based on HPLC traces under our photolysis conditions (Figure 1b). Photocleavage of both caged oligodeoxynucleotides satisfied first-order reaction kinetics with $k = 0.358\ min^{-1}$ (**ODN 1**, $R^2 = 0.975$) and $0.293\ min^{-1}$ (**ODN 2**, $R^2 = 0.986$).

Functionalisation of oligodeoxynucleotides with caged 3′-phosphate, amine, acid and thiol moieties: To synthesise 3′-end modified oligonucleotides with different functional groups by using photolabile protecting moieties, new controlled pore glass supports needed to be synthesised. Previously, photolabile solid-phase supports modified with caged acid and amine groups based on 4,5-dimethoxy-2-nitrobenzyl (DMNB) derivatives (CPG-As, Figure 2) have been reported.[36,37,39] The desired oligodeoxynucleotides can be obtained upon photolysis at 365 nm. Here we synthesised a series of new photocleavable solid-phase supports (**CPG 11–14**, Figure 2) based on 2-amino-1-(2-nitrophenyl) ethanol moiety **9**, which can be readily synthesised from *o*-nitroacetophenone in four steps. As shown in Scheme 2, **9** coupled with 4-pentyoic acid to obtain **11a** with the introduction of an alkyne group that is used to couple with azide-functionalised LCAA-CPG through a copper-catalysed click reaction. Product **11a** serves as an intermediate for the preparation of **11b**, **12b**, **13** and **14b** for further reaction with an azide group for 3′-end modification of solid-phase supports. By using these photolabile supports, oligodeoxynucleotides with 3′-phosphate, amine, carboxylic acid and thiol groups were synthesised.

For the phosphate-group modification of oligonucleotides at the 3′ end, only DMTr-Cl was needed for the reaction with a hydroxyl group of **11a** to give **11b**, which can be di-

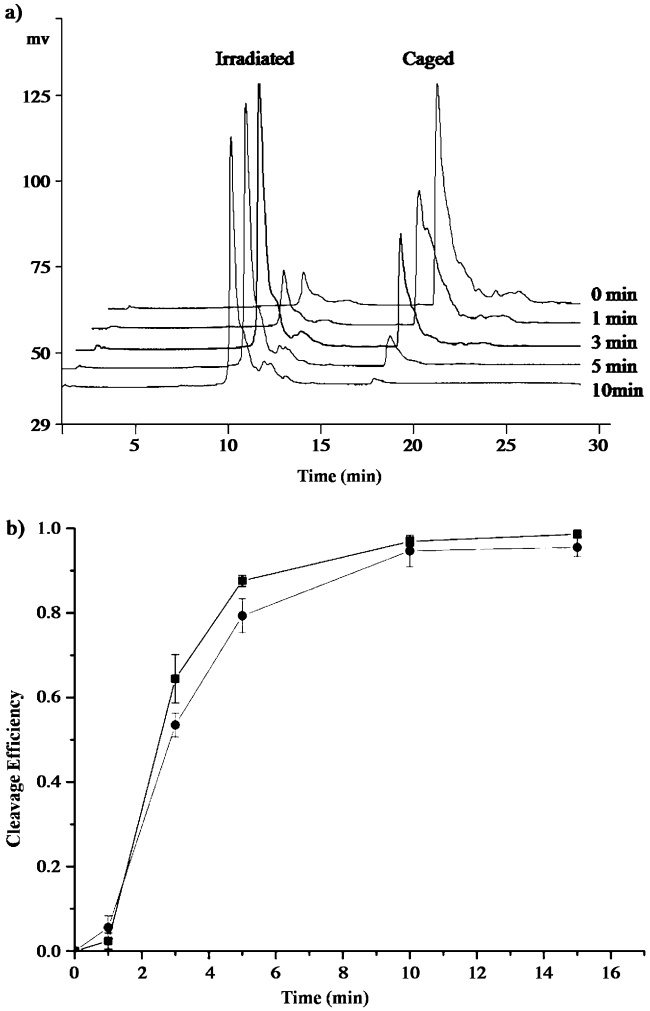


Figure 1. a) HPLC traces of the photocleavage of caged **ODN 2** versus irradiation time. b) Photocleavage efficiencies of **ODN 1** (■) and **ODN 2** (●). The concentration of the oligodeoxynucleotide was 20 nmol mL^{-1} and the photolysis was conducted with UV light (365 nm, 11 m Wcm^{-2}).

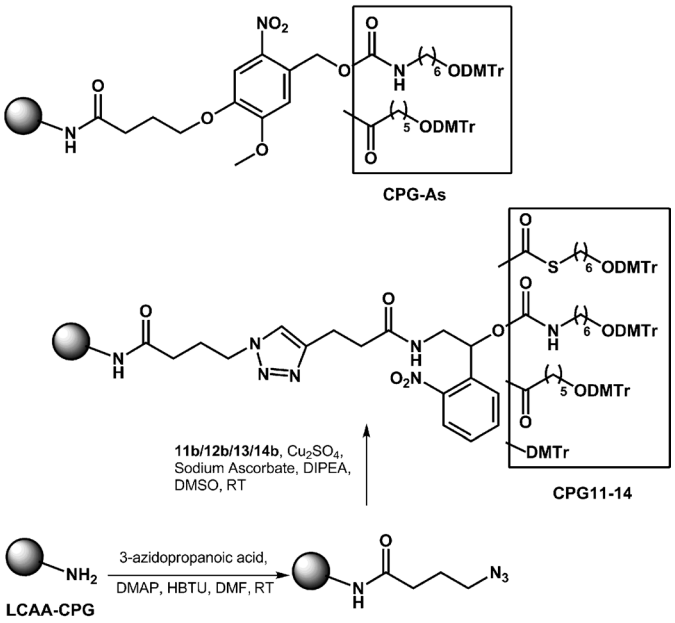
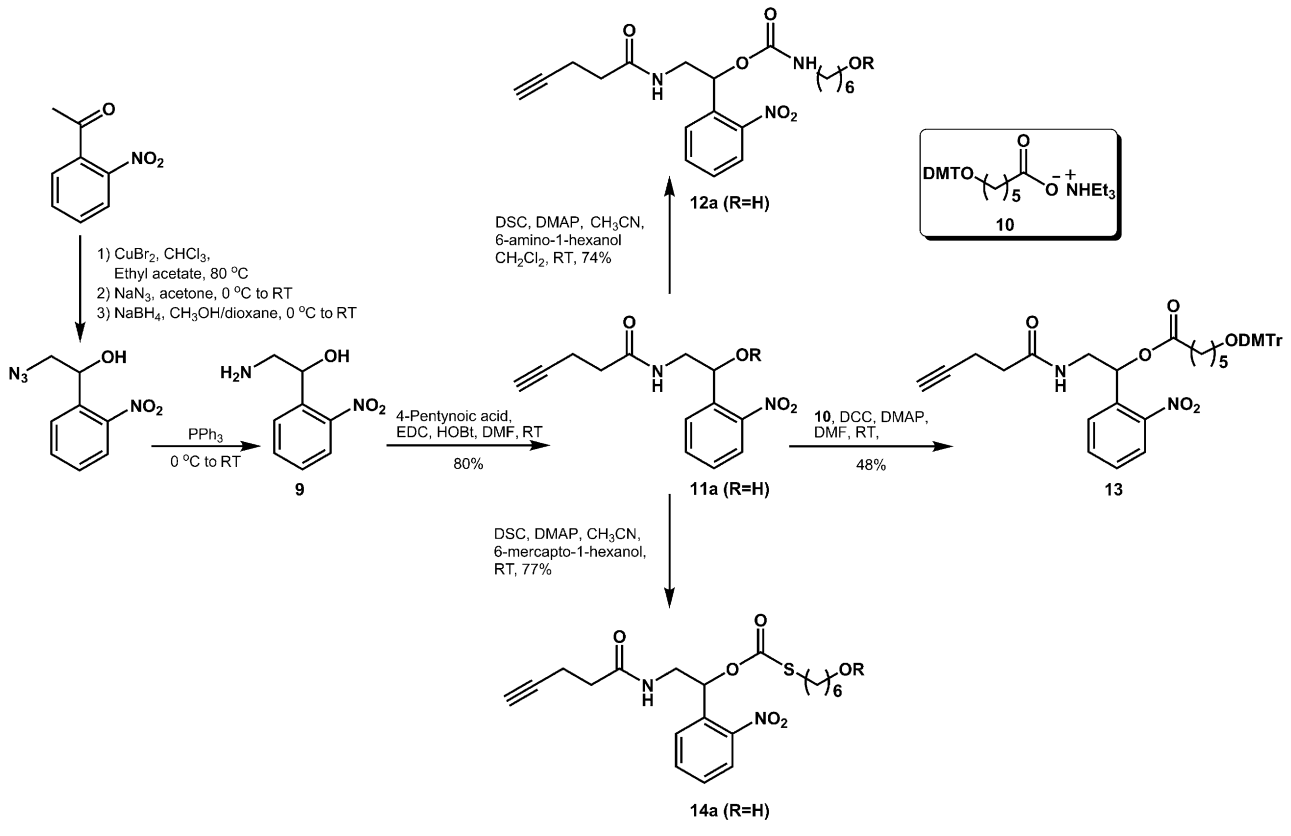


Figure 2. Structure and synthesis of modified CPGs in this study. DIPEA = *N*,*N*-diisopropylethylamine, HBTU = *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate.

rectly used for the synthesis of the photolabile solid support. Similar to the synthesis of **3a**, preparation of **12a** was achieved via reaction of **11a** with DSC, followed by the addition of 6-amino-1-hexanol. Synthesis of **13** was accomplished directly by the coupling of **11a** and **10** with DCC as the coupling reagent. In the case of caged thiol-functionalised CPG, we did not try to synthesise the sulfoether due to its inability to survive the oxidation cycle of DNA synthesis and photoirradiation, as mentioned before. Instead, the carbonothioate **14a** was synthesised by two-step coupling **11a** and 6-mercapto-1-hexanol in one-pot in the presence of DSC. The respective photolabile nitrobenzyl derivatives (**12a**, **14a**) were carried onto **12b** and **14b** by standard dimethoxytritylation.

Solid supports of **CPG 11-14** were prepared by coupling the alkyne group of **11b**, **12b**, **13** and **14b** with the azide preloaded long-chain alkylamine-controlled pore-glass support (LCAA-CPG, 99 μmol g^{-1}), followed by capping with acetic anhydrite (Figure 2). First, LCAA-CPG reacted with 4-azidobutanoic acid in DMF solution at the presence of HBTU and DMAP. Photolabile **11b**, **12b**, **13** and **14b** were then coupled with the azide-loaded CPG through Huisgen cycloaddition. The loading efficiencies were determined by the absorbance of DMTr cation on a certain amount CPG in methylene chloride containing 3% trifluoroacectic acid. The



Scheme 2. Syntheses of photolabile modifiers for modified CPGs. Reagents and conditions for **11a** to **11b**: DMTr-Cl, Py, RT, 78%; for **12**/**14a** to **12**/**14b**: DMTr-Cl, TEA, dichloromethane, RT, 78/76%. DCC = dicyclohexylcarbodiimide, DMTr-Cl: 4,4′-dimethoxytrityl chloride, EDC = 1-ethyl-3-(2-dimethylaminopropyl)carbodiimide, HOBt = *N*-hydroxybenzotriazole.

amount of DMTr was then calculated to prove the effectiveness of two-step coupling (azide loading and click coupling). Loading capacities of these photolabile CPGs over two steps was determined to be over 55 $\mu mol\,g^{-1}$ (see Table S1 in the Supporting Information). To determine the photocleavage efficiencies, a certain amount of these CPGs was irradiated for 30 min in acetonitrile/water, and the DMTr cation of irradiated CPGs and total DMTr cation of the same amount of unirradiated CPGs were then measured. The percentage of lost DMTr cation after photoirradiation was defined as the photocleavage efficiency. Table S1 in the Supporting Information data also lists photocleavage efficiencies for all four photolabile CPGs in 30 min irradiation.

For functionalisation of the 3′-end of oligodeoxynucleotides, 15-mer **ODN 7/8/9/10** (5′-TTT TTC TCT CTC TCT *Y*-3′) were synthesised with the corresponding photolabile CPGs under standard DNA synthesis cycles. The photolysis was conducted on solid-phase suspension (acetonitrile/water v/v 4/1, 6 $mg\,mL^{-1}$) to cleave the oligodeoxynucleotides from the corresponding CPGs. The photocleaved oligodeoxynucleotides were then subject to deprotection with concentrated aqueous ammonium hydroxide. By following HPLC purification and DMTr removal with aqueous acetic acid, identification of the modified oligodeoxynucleotides was confirmed by MS (Table 2).

Labelling modified oligodeoxynucleotides with uncaged functional groups: To prove the activities of uncaged functional groups, different dyes were used for the conjugation with modified oligodeoxynucleotides. **ODN 2**, **ODN 3** and **ODN 8** were selected for labelling reactions. **ODN 2** with the amine group was attached to tetramethylrhodamine (TAMRA) NHS ester in DMF/aqueous $NaHCO_3$, and **ODN 3** with the acid group was coupled with 1-pyrenemethylamine, whereas protected **ODN 8** with the amine group was linked to 1-pyrenebutyric acid. After ammonium hydroxide hydrolysis and HPLC purification, we obtained the expected fluorophore-labelled ODNs with the anticipated MS as listed in Table 2. The labelling efficiencies were usually higher in comparison to the coupling of deprotected oligodeoxynucleotides in aqueous solutions. For **ODN 3**, the efficiency of on-column coupling with pyrenemethylamine by using benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate (BOP) reagent reached 82% based on HPLC results, whereas there was very low yield of the labelled oligodeoxynucleotide product under aqueous conditions due to low solubility of pyrenemethylamine in water. For the 3′-end modified oligodeoxynucleotide **ODN 8**, the uncaged amino group could efficiently couple with 1-pyrenebutyric acid in DMF solution (85% yield), which is better than the yields from oligodeoxynucleotide bioconjugation in aqueous solutions.

Furthermore, circular oligodeoxynucleotides could also be synthesised by using a similar approach. Caged **ODN 11** was synthesised under normal solid-phase synthesis with photolabile acid **CPG 13** as the 3′-end modification and photolabile amine modifier **3b** as the 5′-end modification. The caged **ODN 11** on CPG was irradiated under the same photolysis conditions mentioned above and the supernatant was collected as protected **ODN 11**. After removing all solvents and washing with ether, coupling reagents (BOP, HBTU and EDC) for amide bond formation were added to the residue. The oligodeoxynucleotide was then deprotected with concentrated ammonium hydroxide and was further purified with RP-HPLC. HPLC traces showed two main peaks (see Figure S3 in the Supporting Information). One small peak was confirmed by MS to be the uncyclised linear oligodeoxynucleotide and the other peak with a slightly longer retention time was the expected cyclised oligodeoxynucleotide, the mass of which was confirmed by MS (Table 2). After optimisation of coupling conditions, the combination of BOP/HOBt/DIPEA produced a cyclised product with a better coupling yield (80%) based on integration of peaks corresponding to the linear and circular oligodeoxynucleotides on HPLC traces, as shown in Table S2 in the Supporting Information.

Conclusion

We have developed novel phosphoramidites and CPGs with photolabile protecting groups for modification of oligonucleotides by solid-phase synthesis. We synthesised five photolabile blocks to protect phosphate, amine, thiol, acid and carbonyl groups for the 5′ end of ODNs, and four new photolabile solid-phase CPGs to generate phosphate, amino, thiol and acid groups at the 3′ end of ODNs. To the best of our knowledge, this is the first report of the addition of a carbonyl group at the 5′ end and thiol groups at both ends of ODNs with photolabile modifiers. Furthermore, cyclisation of a protected oligodeoxynucleotide was achieved by using the photolabile amine phosphoramidite modifier and photolabile acid CPG during solid-phase synthesis. These studies provide an alternative way to introduce functional groups into oligonucleotides, and expand the scope of oligonucleotide bio-orthogonal labelling.

Experimental Section

General methods for organic synthesis: All solvents used are dried and distilled by using standard methods. Dichloromethane, TEA, THF and acetonitrile are dried over CaH_2 and are freshly distilled before every use. All reagents were purchased from Alfa Aesar, Sigma Aldrich or J&K and used without further purification. All mixed solvent systems are reported as v/v solutions. All reactions are monitored by TLC using commercial Merck Plates coated with silica gel GF254 (0.24 mm thick). Flash column chromatography is performed with silica gel purchased from Qingdao Haiyang Chemical Company (200–300 mesh). ^1H (400 MHz), ^{13}C (100 MHz) and ^{31}P NMR (162 MHz) spectra are recorded on a Bruker spectrometer at 25 °C. Chemical shifts (δ, ppm) are quoted relative to the residual solvent and coupling constants (J) are corrected and quoted to the nearest 1 Hz. MS are measured on MALDI TOF or a Q-TOF spectrometer by using electrospray ionisation (ESI). When necessary, the reactions are conducted in a dark room.

1-(2-Nitrophenyl) ethanol (1a): 1-(2-Nitrophenyl) ethanol was synthesised according to the literature procedure with minor modification.[51] *o*-Nitroacetophenone (3.51 g, 21.3 mmol) was dissolved in 1,4-dioxane (15 mL) and methanol (9 mL) and stirred for 10 min at 0 °C. Then $NaBH_4$ (1.60 g, 42.3 mmol) was slowly added. The mixture was left to warm to room temperature whilst stirring for 1 h and was then quenched with acetone (15 mL). Solvents were removed in vacuo. The residue was dissolved in ethyl acetate (70 mL) and washed with H_2O (3×50 mL). The organic layer was then dried over Na_2SO_4, filtered and concentrated under reduced pressure to give **1a** as a clear yellow oil (3.52 g, 21.1 mmol, 99% yield); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.84 (dd, 2H, J=8, 11 Hz), 7.63 (t, 1H, J=7 Hz), 7.40 (t, 1H, J=8 Hz), 5.39 (q, 1H, J=6 Hz), 1.54 ppm (d, 3H, J=6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 147.8, 141.0, 133.5, 128.0, 127.5, 124.2, 65.5, 24.2 ppm.

1-(1-Bromoethyl)-2-nitrobenzene (2): 1-(2-Nitrophenyl) ethanol (**1a**) (425 mg, 2.50 mmol), triphenylphosphine (984 mg, 3.75 mmol) and CBr_4 (1.25 g, 3.75 mmol) were dissolved in THF (10 mL) and stirred for 30 min at room temperature. The solution was filtered and concentrated and then purified by flash column chromatography by eluting with 4:1 petroleum ether/ethyl acetate to give **2** as a brown oil (558 mg, 2.43 mmol, 97% yield); ^{1}H NMR (400 MHz, $CDCl_3$) δ=7.88 (dd, 1H, J=1, 4 Hz), 7.81 (dd, 1H, J=1, 4 Hz), 7.65 (dd, 1H, J=1, 4 Hz), 7.42 (dd, 1H, J=1, 7 Hz), 5.80 (q, 1H, J=7 Hz), 2.07 ppm (d, 3H, J=7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ=147.5, 137.7, 133.3, 129.8, 128.8, 124.2, 41.7, 27.1 ppm.

1-(2-Nitrophenyl) ethyl 6-hydroxyhexylcarbamate (3a): 1-(2-Nitrophenyl) ethanol (**1a**) (1.03 g, 6.17 mmol) and DSC (2.37 g, 9.26 mmol) were dissolved in CH_3CN (20 mL). TEA (2.08 mL, 15.0 mmol) was added to the mixture and the solution was left stirring for 3 h at room temperature. 6-Amino-1-hexanol (1.45 g, 12.4 mmol) dissolved in dichloromethane (15 mL) was then added. After stirring for another 30 min at room temperature, the reaction was stopped. The solution was concentrated and the residue was then diluted with dichloromethane (50 mL). The organic layer was washed with H_2O (3×50 mL) and dried over Na_2SO_4. After filtration and concentration, the obtained residue was further purified by flash column chromatography with 1:2 petroleum ether/ethyl acetate as the eluent to give **3a** as a clear yellow oil (1.78 g, 5.74 mmol, 93% yield); ^{1}H NMR (400 MHz, $CDCl_3$) δ=7.90 (d, 1H, J=8 Hz), 7.60 (d, 2H, J= 3 Hz), 7.39(d, 1H, J=4 Hz), 6.20 (d, 1H, J=6 Hz), 4.90 (s, 1H), 3.56 (t, 2H, J=6 Hz), 3.09 (t, 2H, J=6 Hz), 1.83 (s, 1H) 1.59 (d, 3H, J=6 Hz), 1.57–1.24 ppm (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ=155.3, 147.6, 138.7, 133.4, 128.1, 127.0, 124.3, 68.4, 62.5, 40.7, 32.4, 29.8, 26.2, 25.2, 22.2 ppm; MS (ESI-TOF$^+$): m/z: calcd for $C_{15}H_{22}N_2O_5$: 333.14; found: 333.10 $[M]^+$.

6-(*tert*-Butyldimethylsilyloxy)hexanoic acid (4): *tert*-Butyldimethylsilyl chloride (1.84 g, 12.2 mmol) and imidazole (800 mg, 11.8 mmol) were dissolved in DMF (10 mL). Ethyl 6-hydroxyhexanoate (650 μL, 4.00 mmol) was added to the mixture with stirring under nitrogen at room temperature. After 24 h, TLC confirmed the reaction was completed. The reaction mixture was then diluted with ether (50 mL). The organic layer was washed with H_2O (3×50 mL) and dried over Na_2SO_4. The solution was then concentrated to give the residue as a yellow oil, which was then dissolved in a methanol solution of Triton B (40% w/w, 10 mL) and stirred for 1 h at room temperature. Methanol was then removed and the residue was diluted with H_2O (20 mL), followed by the adjustment of solution acidity to pH 4 with 1 M HCl. The aqueous phase was extracted by ether (5×20 mL) and the combined ether solution was dried over Na_2SO_4. Ether was removed to give the residue **4** as a yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ=3.60 (t, 2H, J=6 Hz), 2.36 (t, 2H, J= 7 Hz), 1.65 (m, 2H), 1.53 (m, 2H), 1.40 (dd, 2H, J=4, 6 Hz), 0.89 (s, 9H), 0.04 ppm (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=179.1, 62.9, 33.9, 32.4, 26.0, 25.3, 24.5, 18.3, −5.3 ppm.

1-(2-Nitrophenyl) ethyl 6-hydroxyhexanoate (5a): 1-(1-Bromoethyl)-2-nitrobenzene (**2**), Cs_2CO_3 (1.30 g, 3.99 mmol) and NaI (1.20 g, 8.00 mmol) were dissolved in acetone (15 mL). *tert*-Butyldimethylsilyl chloride (**4**) (1.25 g, 5.42 mmol) dissolved in anhydrous acetone (20 mL) was added to the mixture with stirring. After refluxing for 16 h, the reaction was stopped and the solvent was removed in vacuo. The residue was dissolved in ether (80 mL) and the ether solution was washed with H_2O (3× 80 mL) and dried over Na_2SO_4. After removal of ether, the residue was purified by flash column chromatography with 10:1 petroleum ether/ ethyl acetate as the eluent to give yellow oil [1-(2-nitrophenyl) ethyl 6-(*tert*-butyldimethylsilyloxy)hexanoate], which was further dissolved in TBAF (1 M in THF, 20 mL). After stirring for 1 h at room temperature, the solution was concentrated and the obtained residue was purified by flash column chromatography with 1:1 petroleum ether/ethyl acetate as the eluent to give **5a** as a clear, yellow oil (748 mg, 2.66 mmol, 66% yield for 3 steps from ethyl 6-hydroxyhexanoate). ^{1}H NMR (400 MHz, $CDCl_3$) δ=7.92 (d, 1H, J=8 Hz), 7.62 (d, 2H, J=4 Hz), 7.42 (m, 1H), 6.32 (m, 1H), 3.62 (t, 2H, J=6 Hz), 2.34 (m, 2H), 1.64 (d, 3H, J=3 Hz), 1.62–1.32 ppm (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ=172.4, 138.0, 133.4, 128.3, 127.1, 124.4, 67.9, 62.6, 34.2, 32.2, 25.2, 24.5, 21.9 ppm; MS (ESI-TOF$^+$): m/z: calcd for $C_{14}H_{21}NO_3$: 304.12; found: 304.08 $[M]^+$.

6-[1-(2-Nitrophenyl)ethylthio]hexan-1-ol (6a): Cs_2CO_3 (438 mg, 1.34 mmol), TBAB (432 mg, 1.34 mmol) and NaI (227 mg, 1.51 mmol) were dissolved in DMF (6 mL) under nitrogen. 6-Mercapto-1-hexanol (183 μL, 2.07 mmol) was added to above solution and the mixture was stirred for 1 h at room temperature and was then cooled to 0 °C. 1-(1-Bromoethyl)-2-nitrobenzene (**2**) (302 mg, 1.31 mmol) dissolved in DMF (5 mL) was added dropwise. After stirring for 11 h, the reaction mixture was diluted in ethyl acetate (50 mL) and the ethyl acetate solution was then washed with H_2O (3×50 mL). The combined organic solution was dried over Na_2SO_4. After the removal of ethyl acetate, the residue was purified by flash column chromatography with 2:1 petroleum ether/ethyl acetate as the eluent to give **6a** as a clear yellow oil (297 mg, 1.05 mmol, 80% yield); ^{1}H NMR (400 MHz, $CDCl_3$) δ=7.81 (d, 1H, J=8 Hz), 7.69 (d, 1H, J=8 Hz), 7.56 (t, 1H, J=8 Hz), 7.32 (t, 1H, J=8 Hz), 4.55 (q, 1H, J=7 Hz), 3.55 (t, 2H, J=7 Hz), 2.29 (m, 2H), 1.56 (d, 3H, J=7 Hz), 1.45 (m, 4H), 1.26 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=149.3, 139.1, 132.8, 129.4, 127.4, 123.5, 62.6, 38.1, 32.4, 31.5, 29.2, 28.4, 25.1, 22.8 ppm; MS (ESI-TOF$^+$): m/z: calcd for $C_{14}H_{21}NO_3S$: 306.11; found: 306.08 $[M]^+$.

(*S*)-6-Hydroxyhexyl *O*-1-(2-nitrophenyl)ethyl carbonothioate (7a): 1-(2-Nitrophenyl) ethanol (**1a**) (1.28 g, 7.66 mmol), DSC (2.94 g, 11.5 mmol) and TEA (1.59 mL, 11.5 mmol) were dissolved in acetonitrile (20 mL) under nitrogen. After stirring at room temperature for 1 h, DMAP (1.40 g, 11.5 mmol) and 6-mercapto-1-hexanol (1.45 mL, 11.5 mmol) were added. H_2O (80 mL) was added to stop the reaction over another 15 min whilst stirring. The water layer was exacted with dichloromethane (3× 50 mL). The organic phase was collected, dried and concentrated. The residue was further purified by flash column chromatography with 2:1 petroleum ether/ethyl acetate as the eluent to give **7a** as a clear yellow oil (1.32 g, 4.04 mmol, 57% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.94 (d, 1H, J=8 Hz), 7.64 (d, 2H, J=4 Hz), 7.42 (m, 1H), 6.41 (q, 1H, J= 6 Hz), 3.58 (t, 2H, J=6 Hz), 2.78 (m, 2H), 1.78 (br s, 1H), 1.65 (d, 3H, J=7 Hz), 1.60–1.49 (m, 4H), 1.33 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=170.3, 147.2, 137.4, 133.8, 128.5, 127.0, 124.4, 70.9, 62.5, 32.3, 30.7, 29.5, 28.2, 25.1, 22.0 ppm; MS (ESI-TOF): m/z: calcd for $C_{14}H_{21}NO_3S$: 350.10; found: 350.15 $[M]^+$.

4-Hydroxybutan-2-one 1-(2-nitrophenyl)-1,2-diyl ketal (8a): A THF solution (15 mL) of 1-(2-nitrophenyl)ethane-1,2-diol (**7**, 183 mg, 1.0 mmol, prepared by following a previous report[29,52]), 4-hydroxybutan-2-one (86 μL, 1.0 mmol) and *p*-toluenesulfonic acid (3.8 mg, 0.02 mmol) was refluxed with molecular sieves in a Dean–Stark setup for 6 h. The reaction solution was filtered and concentrated. The obtained residue was then purified by flash column chromatography by eluting with 3:1 petroleum ether/ethyl acetate to give **8a** as a yellow oil (100 mg, 0.4 mmol, 40% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.07 (d, 1H, J=8 Hz), 7.95 (d, 1H, J=8 Hz), 7.69 (t, 1H, J=8 Hz), 7.47 (dt, 1H, J=1,8 Hz), 5.66 (t, 1H, J=7 Hz), 4.75 (dd, 0.1H, J=7, 8 Hz), 4.68 (dd, 0.9H, J=7,8 Hz), 3.96 (m, 1H), 3.86 (m, 1H), 3.76 (m, 1H), 2.18 (m, 2H), 1.61 (s, 0.4H), 1.50 ppm (s, 2.6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=147.3, 136.5, 134.2, 128.6, 127.6, 127.4, 124.8, 111.4, 73.7, 71.2, 59.0, 41.1, 23.0 ppm; MS (ESI-TOF$^+$): m/z: calcd: $C_{15}H_{22}N_2O_5$: 276.08; found: 276.06 $[M]^+$.

General procedure for 1b, 3b, 5b–8b: Alcohol (**1a**, **3a**, **5a**, **6a**, **8a**) (0.5 mmol, 1.0 equiv) was weighted into a round-bottomed flask. The

flask was purged with nitrogen and dichloromethane (10 mL) and triethylamine (3.0 equiv) were added to yield a clear colourless solution, followed by the addition of 2-cyanoethyl diisopropylchlorophosphoramidite (1.5 equiv). The reaction mixture was stirred at room temperature. After disappearance of the alcohol, the reaction was stopped. The reaction solution was then diluted with dichloromethane and washed with 0.1 M $NaHCO_3$ (2×30 mL). The organic layer was collected and dried over Na_2SO_4. The solvent was removed and the residue was purified by flash column chromatography with petroleum ether/dichloromethane containing 3% triethylamine as the eluent to give **1b**, **3b**, **5b**, **6b**, **8b** as a yellow oil.

*Product **1b***: Yield: 71%; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.80 (m, 2H), 7.59 (m, 1H), 7.35 (m, 1H), 5.46 (m, 1H), 3.81 (m, 1H), 3.60 (m, 2H), 3.45 (m, 1H), 2.62–2.46 (m, 2H), 1.52 (m, 3H), 1.15–0.86 ppm (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=146.9 (m), 140.0 (m), 133.2 (m), 128.2 (m), 127.6 (m), 123.6 (m), 117.2 (m), 67.0 (dd, J=10 Hz), 58.2 (dd, J=20 Hz), 42.9 (m), 24.9–24.0 (m), 23.8 (m), 19.9 ppm (m); ^{31}P NMR (162 MHz, $CDCl_3$): δ=147.7 (s), 147.3 ppm (s).

*Product **3b***: Yield: 59%; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.92 (d, 1H, J=8 Hz), 7.61 (d, 2H, J=4 Hz), 7.40 (m, 1H), 6.22 (q, 1H, J=6 Hz), 4.75 (br, 1H), 3.82 (m, 2H), 3.58 (m, 4H), 3.12 (m, 2H), 2.63 (t, 2H), 1.60 (d, 3H, J=6 Hz), 1.58 (m, 2H), 1.47 (m, 2H), 1.34 (m, 4H), 1.17 ppm (t, 12H, J=4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ=155.2, 147.5, 138.7, 133.3, 128.0, 127.0, 124.2, 117.6, 68.3, 63.3 (d, J=17 Hz), 58.1 (d, J=19 Hz), 42.9 (d, J=12 Hz), 40.8, 30.9 (d, J=7 Hz), 29.7, 26.2, 25.4, 24.4 (d, J=8 Hz), 22.1, 20.2 ppm (d, J=7 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ=147.2 ppm (s).

*Product **5b***: Yield: 63%; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.91 (d, 1H, J=8 Hz), 7.61 (d, 2H, J=4 Hz), 7.42 (m, 1H), 6.30 (t, 1H, J=6 Hz), 3.85–3.75 (m, 2H), 3.66–3.53 (m, 4H), 2.62 (t, 2H, J=6 Hz), 2.32 (m, 2H), 1.64–1.34 (m, 11H), 1.16 ppm (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=172.3, 147.8, 138.0, 133.4, 128.3, 127.1, 117.6, 67.8, 63.4, 63.2 (d, J=17 Hz), 58.2 (d, J=19 Hz), 43.0(d, J=12 Hz), 34.2, 30.8 (d, J=8 Hz), 25.4, 24.5 (d, J=8 Hz), 21.9, 20.3 ppm (d, J=7 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ=147.3 ppm (s).

*Product **6b***: Yield: 54%; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.84 (d, 1H, J=8 Hz), 7.71 (d, 1H, J=8 Hz), 7.58 (t, 1H, J=8 Hz), 7.34 (t, 1H, J=8 Hz), 4.56 (q, 1H, J=7 Hz), 3.80 (m, 2H), 3.58 (m, 4H), 2.62 (t, 2H, J=6 Hz), 2.30 (m, 2H0), 1.59 (d, 3H, J=7 Hz), 1.54 (m, 2 Hz), 1.44 (m, 2 Hz), 1.28 (m, 4 Hz), 1.16 ppm (t, 12H, J=7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ=149.4, 139.2, 132.8, 129.4, 127.5, 123.6, 117.6, 63.5 (d, J=17 Hz), 58.3 (d, J=18 Hz), 43.0 (d, J=12 Hz), 38.2, 31.6, 30.9 (d, J=7 Hz), 29.1, 28.4, 25.4, 24.5 (t, J=8 Hz), 22.9, 20.3 ppm (d, J=7 Hz); ^{31}P NMR (162 MHz, $CDCl_3$): δ=147.3 ppm (s).

*Product **7b***: Yield: 73%; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.95 (d, 1H, J=8 Hz), 7.64 (d, 2H, J=4 Hz), 7.43 (m, 1H), 6.40 (q, 1H, J=8 Hz), 3.80 (m, 2H), 3.58 (m, 4H), 2.77 (m, 2H), 2.61 (t, 2H, J=10 Hz), 1.65 (d, 3H, J=8 Hz), 1.57 (m, 4H), 1.34 (m, 2H), 1.15 ppm (t, 12H, J=8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ=170.3, 147.3, 137.5, 133.8, 128.5, 127.1, 124.4, 117.6, 70.9, 63.4 (d, J=17 Hz), 58.2 (d, J=18 Hz), 42.9 (d, J=12 Hz), 30.9 (d, J=7 Hz), 30.8, 29.5, 28.2, 25.3, 24.5 (t, J=8 Hz), 24.4, 22.0, 20.3 ppm (d, J=7 Hz); ^{31}P NMR (162 MHz, $CDCl_3$): δ=147.2 ppm (s).

*Product **8b***: Yield: 74%; ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.04 (d, 1H, J=8 Hz), 7.92 (d, 1H, J=8 Hz), 7.67 (t, 1H, J=8 Hz), 7.45(t, 1H, J=8 Hz), 5.59 (m, 1H), 4.60 (t, 1H, J=8 Hz), 3.88–3.59 (m, 7H), 2.64 (t, 2H, J=3 Hz), 2.24 (t, 0.9H, J=7 Hz), 2.12 (t, 1.1H, J=7 Hz), 1.60 (s, 1.7H), 1.48 (s, 1.3H), 1.19 ppm (dd, 12H, J=4,6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ=147.3, 137.0, 136.6, 134.0, 128.4, 127.6, 124.6, 117.5, 110.5, 110.2, 74.3, 71.3, 59.6, 58.3, 43.1, 40.9, 39.5, 24.6, 23.5, 20.3 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ=147.7 ppm (s).

2-Amino-1-(2-nitrophenyl) ethanol (9): 2-Amino-1-(2-nitrophenyl) ethanol (**9**) was synthesised according to the protocol published before with modification[53] from *o*-nitroacetophenone as a faint yellow solid (40% yield over 3 steps). ^{1}H NMR (400 MHz, [D$_4$]MeOH) δ=7.80 (dd, 2H, J=8,11 Hz), 7.62 (t, 1H, J=7 Hz), 7.40 (t, 1H, J=8 Hz), 5.06 (d, 1H, J=3,8 Hz), 2.90 (dd, 1H, J=2,13 Hz), 2.64 ppm (dd, 1H, J=8,13 Hz); ^{13}C NMR (100 MHz, [D$_4$]MeOH) δ=149.4, 139.7, 134.3, 129.5, 129.4, 125.1, 49.6 ppm; MS (ESI-TOF$^+$): m/z: calcd for $C_8H_{10}N_2O_3$: 205.06; found: 205.06 $[M]^+$.

Triethylammonium 6-[bis(4-methoxyphenyl)(phenyl)methoxy]hexanoate (10): Ethyl 6-hydroxyhexanoate (200 μL, 1.23 mmol) and triethylamine (430 μL, 3.08 mmol) were added to a solution of DMTr-Cl (500 mg, 1.48 mmol) in dichloromethane (10 mL). After stirring at room temperature for 1 h, the reaction mixture was washed with saturated aqueous $NaHCO_3$ (2×30 mL). The combined organic layer was dried over Na_2SO_4. The solvent was removed and the residue was then dissolved in the mixture of NaOH aqueous solution (6 mol L^{-1}, 25 mL) and ethanol (4 mL). The mixture was stirred at room temperature and the reaction was monitored by the disappearance of the ethyl ester by TLC analysis. After completion, the mixture was diluted with $CHCl_3$ (50 mL) and washed with water (3×50 mL). The organic layer was concentrated and the residue was purified by flash column chromatography with 3:7:1 methanol/dichloromethane/triethylamine as the eluent to give **10** as a white solid (550 mg, 1.08 mmol, 87% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.42–7.18 (m, 10H), 6.80 (d, 4H, J=9 Hz), 3.78 (s, 6H), 3.47 (s, 1H), 3.01 (t, 2H, J=7 Hz), 2.84 (q, 6H, J=7 Hz), 2.21 (t, 2H, J=8 Hz), 1.60 (m, 4H, J=8 Hz), 1.38 (dd, 2H, J=7,12 Hz), 1.16 ppm (t, 3H, J=7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ=179.2, 158.3, 145.5, 136.8, 130.2, 128.2, 127.6, 126.5, 113.0, 85.6, 63.6, 55.2, 45.1, 36.5, 30.1, 26.4, 26.0, 9.5 ppm.

***N*-[2-Hydroxy-2-(2-nitrophenyl)ethyl]-4-pentynamide (11a)**: 4-Pentynoic acid (640 mg, 6.51 mmol), EDC·HCl (1.24 g, 6.47 mmol) and HOBt (996 mg, 6.50 mmol) were dissolved in DMF (5 mL) and stirred at room temperature for 1 h. Then **9** (988 mg, 5.40 mmol) in DMF (3 mL) was added dropwise. The reaction mixture was stirred at room temperature for another 3 h and was then diluted with ethyl acetate (50 mL). The ethyl acetate solution was washed with H_2O (3×50 mL) and dried over Na_2SO_4. The solution was concentrated and the residue was purified by flash column chromatography with 1:1 petroleum ether/ethyl acetate as the eluent to give **11a** as a faint yellow solid (1.13 g, 4.31 mmol, 80% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.95 (m, 2H), 7.67(m, 1H), 7.45(m, 1H, J=1, 8 Hz), 6.22 (s, 1H), 5.38 (t, 1H, J=4 Hz), 3.70 (dd, 2H, J=6 Hz). 2.55 (m, 2H), 2.46 (m, 2H), 2.02 ppm (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=173.8, 147.7, 137.5, 133.6, 128.8, 128.5, 124.5, 82.6, 70.8, 69.7, 47.8, 35.1, 14.9 ppm; MS (ESI-TOF$^+$): m/z: calcd for $C_{13}H_{14}N_2O_4$: 285.09; found: 285.08 $[M]^+$.

***N*-{2-[Bis(4-methoxyphenyl)(phenyl)methoxy]-2-(2-nitrophenyl)ethyl}-4-pentynamide (11b)**: *N*-[2-Hydroxy-2-(2-nitrophenyl)ethyl]-4-pentynamide (**11a**) (92 mg, 351 μmol) and DMTr-Cl (150 mg, 443 μmol) were dissolved in pyridine (5 mL) and the reaction mixture was stirred at room temperature overnight. The solvent was removed and the residue was purified by flash column chromatography with 1:1 petroleum ether/ethyl acetate containing 1% triethylamine as the eluent to give **11b** as a colourless oil (154 mg, 273 μmol, 78% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ=7.77 (d, 1H, J=8 Hz), 7.66 (d, 1H, J=8 Hz), 7.49–7.18 (m, 11H), 6.68 (dd, 4H, J=9 Hz), 5.38 (t, 1H, J=5 Hz), 3.78 (d, 6H, J=8 Hz), 2.43 (m, 2H), 2.32 (m, 2H), 1.96 ppm (t, 1H, J=2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ=170.5, 158.6, 158.5, 146.8, 145.1, 138.1, 135.6, 135.3, 132.7, 130.2, 130.1, 130.0, 127.9, 127.8, 127.3, 126.9, 123.7, 113.2, 113.0, 87.6, 82.9, 69.8, 69.2, 46.2, 45.1, 35.3, 14.6 ppm; MS (ESI-TOF$^+$): m/z: calcd for $C_{34}H_{32}N_2O_6$: 587.22; found: 587.26 $[M]^+$.

1-(2-Nitrophenyl)-2-(4-pentynamido)ethyl 6-hydroxyhexylcarbamate (12a): *N*-[2-Hydroxy-2-(2-nitrophenyl)ethyl]-4-pentynamide (**11a**) (131 mg, 500 μmol), DSC (154 mg, 600 μmol) and DMAP (74 mg, 600 μmol) were dissolved in CH_3CN (7 mL) under nitrogen. The mixture was stirred at room temperature for 30 min. The solution of 6-amino-1-hexanol (70 mg, 600 μmol) in dichloromethane (5 mL) was then added dropwise. After stirring at room temperature for another 10 min, the mixture was concentrated and the residue was purified by flash column chromatography with 10:1 dichloromethane/triethylamine as the eluent to give **12a** as a yellow solid (150 mg, 370 μmol, 74% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.93 (d, 1H, J=8 Hz), 7.63 (m, 2H), 7.42 (m, 1H), 6.65 (br, 1H), 6.19 (m,1H), 5.50 (br, 1H), 3.85 (m, 1H), 3.55 (m, 3H), 3.05 (dd, 2H, J=6,12 Hz), 2.49 (s, 1H), 2.43 (m, 2H), 2.35 (d, 2H, J=14 Hz), 1.96 (s, 1H), 1.51–1.41 (m, 4H), 1.31–1.28 ppm (m, 4H);

^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.4, 155.4, 134.4, 133.5, 128.8, 128.0, 124.6, 82.6, 71.0, 69.2, 62.3, 43.8, 40.8, 35.1, 32.3, 29.5, 26.2, 25.2, 14.7 ppm; MS (ESI-TOF$^+$): *m/z*: calcd for $C_{20}H_{27}N_3O_6$: 428.18; found: 428.13 $[M]^+$.

1-(2-Nitrophenyl)-2(4-pentynamido)ethyl 6-[bis(4-methoxyphenyl)-(phenyl) methoxy]hexylcarbamate (12b): 1-(2-Nitrophenyl)-2-(4-pentynamido)ethyl 6-hydroxyhexylcarbamate (**12a**) (97 mg, 240 μmol) and DMTr-Cl (100 mg, 261 μmol) were dissolved in dichloromethane (5 mL) under nitrogen to yield a purplish-red solution. Triethylamine (82 μL, 590 μmol) was added and the mixture was stirred at room temperature for 30 min to generate a yellow solution. The reaction mixture was diluted with dichloromethane (40 mL) and the organic solution was then washed with saturated $NaHCO_3$ solution (2×50 mL). The combined dichloromethane solution was dried over Na_2SO_4. The solvent was removed and the residue was purified by flash column chromatography with 1:1 petroleum ether/ethyl acetate containing 1% triethylamine as the eluent to give **12b** as a colourless foam (127 mg, 187 μmol, 78% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.97–7.17 (m, 13H), 6.81 (d, 4H, *J* = 9 Hz), 6.26 (br, 1H), 6.16 (m, 1H), 4.92 (br, 1H), 3.89 (m, 1H), 3.78 (s, 6H), 3.70 (m, 1H), 3.09 (m, 2H), 3.02 (m, 2H, *J* = 6 Hz), 2.47 (t, 2H, *J* = 6 Hz), 2.37 (t, 2H, *J* = 7 Hz), 1.96 (d, 1H, *J* = 2 Hz), 1.72 (s, 1H), 1.57 (t, 2H, *J* = 7 Hz),1.44 (t, 2H, *J* = 7 Hz), 1.35 (t, 2H, *J* = 7 Hz), 1.25 ppm (t, 3H, *J* = 7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.0, 158.3, 155.2, 145.4, 136.7, 133.6, 130.0, 129.0, 128.2, 128.0, 127.7, 126.6, 124.7, 113.0, 85.6, 82.8, 71.1, 69.3, 63.2, 55.2, 44.0, 41.1, 35.3, 29.9, 29.7, 26.6, 25.9, 14.8, 14.2 ppm; MS (ESI-TOF$^+$): *m/z*: calcd for $C_{41}H_{45}N_3O_8$: 730.31; found: 730.21 $[M]^+$.

1-(2-Nitrophenyl)-2-(4-pentynamidoethyl 6-[bis(4-methoxyphenyl)-(phenyl)methoxy] hexanoate (13): Triethylammonium 6-[bis(4-methoxyphenyl)(phenyl)methoxy] hexanoate (**10**) (270 mg, 504 μmol), DCC (210 mg, 800 μmol) and DMAP (18 mg, 80 μmol) were dissolved in DMF. After stirring at room temperature for 1 h, **11a** (100 mg, 382 μmol) was added and the reaction mixture was further stirred at 70 °C for another 3 h. After the reaction was stopped, the reaction mixture was diluted with ethyl acetate (50 mL). The organic layer was washed with H_2O (3×50 mL) and dried over Na_2SO_4. After removal of solvent, the residue was purified by flash column chromatography with 1:1 petroleum ether/ethyl acetate containing 1% triethylamine as the eluent to give **13** as a yellow oil (121 mg, 185 μmol, 48% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.90–7.08 (m, 13H), 6.73 (d, 4H, *J* = 8 Hz), 6.28 (m, 1H), 6.00 (br, 1H), 4.03 (q, 1H, *J* = 7 Hz), 3.82 (m, 1H), 3.70 (s, 6H), 2.39–2.23 (m, 6H), 1.96 (s, 2H), 1.88 (s, 1H), 1.53–1.25 ppm (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.6, 170.9, 158.3, 148.0, 145.3, 136.5, 133.6, 133.5, 129.9, 129.1, 129.0, 128.1, 128.0, 127.8, 127.7, 127.6, 126.5, 124.6, 113.1, 112.9, 85.6, 82.8, 70.2, 69.3, 63.0, 60.3, 55.1, 43.5, 35.1, 34.0, 25.8, 25.1, 24.6, 24.3, 14.7, 14.1 ppm; MS (ESI-TOF$^+$): *m/z*: calcd for $C_{40}H_{42}N_2O_8$: 701.28; found: 701.21 $[M]^+$.

(*S*)-6-Hydroxyhexyl-*O*-1-(2-nitrophenyl)-2-(4-pentynamido)ethylcarbonothioate (14a): *N*-[2-Hydroxy-2-(2-nitrophenyl)ethyl]-4-pentynamide (**11a**) (199 mg, 400 μmol), DSC (123 mg, 480 μmol) and DMAP (60 mg, 480 μmol) were dissolved in acetonitrile (10 mL) under nitrogen. The mixture was stirred at room temperature for 30 min and then 6-mercapto-1-hexanol (65 μL, 480 μmol) was added. After another 1.5 h, the mixture was concentrated and the residue was purified by flash column chromatography with 1:1 petroleum ether/ethyl acetate containing 1% triethylamine as the eluent to give **14a** as a yellow oil (123 mg, 292 μmol, 77% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.01 (d, 1H, *J* = 8 Hz), 7.65 (m, 2H), 7.48 (dd, 1H, *d* = 2,4 Hz), 6.45 (dd, 1H, *J* = 4,7 Hz), 6.18 (br, 1H), 3.90–3.79 (m, 2H), 3.62 (dd, 2H, *J* = 2,7 Hz), 2.81 (t, 2H, *J* = 7 Hz), 2.44 (m, 2H), 2.37 (m, 2H), 1.72–1.45 ppm (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 171.0, 170.7, 147.7, 133.8, 133.1, 129.2, 127.9, 124.9, 82.8, 73.2, 69.4, 62.7, 43.3, 39.0, 35.2, 32.5, 31.0, 29.4, 28.2, 29.2, 25.5, 14.7 ppm; MS (ESI-TOF$^+$): *m/z*: calcd for $C_{20}H_{26}N_2O_6S$: 445.14; found: 445.15 $[M]^+$.

(*S*)-6-[Bis(4-methoxyphenyl)(phenyl)methoxy]hexyl-*O*-1-(2-nitrophenyl)-2-(4-pentynamido)ethyl carbonothioate (14b): (*S*)-6-[Bis(4-methoxyphenyl)(phenyl)methoxy]hexyl-*O*-1-(2-nitrophenyl)-2-(4-pentynamido)ethyl carbonothioate (**14b**) was obtained by a similar procedure as **12a** to **12b** as a yellow oil (76% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.03–7.25 (m, 13H), 6.81 (d, 4H, *J* = 9 Hz), 6.47 (dd, 1H, *J* = 4,7 Hz), 6.08 (br, 1H), 3.88–3.81 (m, 2H), 3.77 (s, 6H), 3.02 (t, 2H, *J* = 7 Hz), 2.78 (t, 2H, *J* = 7 Hz), 2.45 (t, 2H, *J* = 2 Hz), 2.44 (m, 2H), 1.99 (t, 2H, *J* = 3 Hz), 1.59–1.55 (m, 4H), 1.35–1.30 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 170.8, 170.6, 158.3, 147.7, 145.3, 136.6, 133.7, 133.2, 130.0, 129.2, 128.1, 127.9, 127.6, 126.5, 124.9, 112.9, 85.6, 82.8, 73.1, 69.5, 63.1, 55.1, 43.3, 35.2, 31.1, 29.8, 29.5, 28.5, 25.7, 14.7 ppm; MS (ESI-TOF$^+$): *m/z*: calcd for $C_{41}H_{44}N_2O_8S$: 747.28; found: 747.27 $[M]^+$.

Modification for LCAA-CPG: HBTU (38 mg, 100 μmol), DMAP (12 mg, 100 μmol) and 4-azidobutanoic acid (43 mg, 330 μmol) were dissolved in DMF (1 mL). The mixture was shaken for 30 min and then added to LCAA-CPG (100 mg, 99 μmol g^{-1}, 9.9 μmol). The mixture was shaken at room temperature for 24 h. Afterwards the solid phase was washed with methanol (3×1 mL), dichloromethane (3×1 mL) and ether (1 mL) sequentially. The solid phase was then dispersed in DMSO (1 mL) with **11b/12b/13/14b** (200 μmol), L-ascorbic acid sodium salt (20 mg, 100 μmol), copper(II) sulfate (8 mg, 50 μmol) and DIPEA (8 μL, 50 μmol). The mixture was stirred at 25 °C for 24 h. The solid phase was washed with DMSO (1 mL), 0.1 M $NaHCO_3$ aq (1 mL), methanol (3×1 mL), dichloromethane (3×1 mL) and ether (1 mL). The beads were capped with Cap A and Cap B for 30 min at room temperature.

Loading efficiency: Modified CPGs were treated with 3% TFA in dichloromethane for 3 min at room temperature for deblocking. The loading efficiency was calculated by the amount of DMTr cation in dichloromethane ($\lambda_{obs.}$ = 504 nm) relative to the amino group on LCAA-CPG purchased.

Cleavage efficiency: Modified CPGs were irradiated for 30 min (365 nm, 11 mWcm^{-2}) in acetonitrile/water (v/v 4/1, 6 mg mL^{-1}) while stirring. The solid phase was dried and the solution phase was concentrated in vacuo. The cleavage efficiency was determined by the amount of DMTr cation cleaved relative to the total DMTr on CPG.

General method for oligonucleotide synthesis: Oligonucleotides were synthesised by using an Applied Biosystems Incorporated 394 automated synthesiser. Long-chain alkylamine controlled pore glass support was purchased from Millipore Corporation. CPG:dG and phosphoramidite monomers were purchased from AuGCT Biotech. All oligodeoxynucleotides were synthesised on a 1 μmol scale with standard DNA synthesis cycles on CPG:dG (**ODN 1–6**) or modified LCAA-CPG (**ODN 7–11**). For the terminal coupling of **ODN 1–6**, the coupling time was extended to 240 s.

Semi-preparation HPLC was carried out with Agilent C18 column (5 μm, 9.4×250 mm) on Varian Prostar. Conditions: solvent A, 0.05 M TEAA buffer; solvent B, acetonitrile. Started at 15% B; linear gradient to 50% B over 35 min, flow rate: 1 mL min^{-1}. HPLC analyses were performed with Symmetry C18 column (5 μm, 9.4×250 mm) on Waters alliance e2695 started at 0% B; linear gradient to 30% B over 30 min. MS was measured either on ESI-Q-TOF or MALDI-TOF.

Photolysis: All samples were irradiated in a quartz cell with a stirrer bar by UVP high intensity inspection UV Lamp (11 mWcm^{-2}) at 365 nm for 30 min or less. The irradiated samples were maintained at ≤30 °C with an ice bag.

Hydrolysis: Concentrated ammonium hydroxide (500 μL) was added to dry ODNs or CPGs, shaken for 24 h at room temperature, and was then concentrated by Thermo Savant SPD 2010 SpeedVac System.

DMTr removal: 80% aqueous acetic acid (500 μL) was added to dry ODNs, shaken for 30 min at room temperature, and was then concentrated.

Attachment ODN 2 with 5-TAMRA-NHS: **ODN 2** on CPG was suspended in acetonitrile/water (v/v 4/1, 6 mg mL^{-1}) in a quartz cell. After 365 nm UV irradiation for 30 min, the CPG was washed with acetonitrile, dried and suspended in DMF (500 μL) and 0.1 M $NaHCO_3$, followed by the addition of 5-TAMRA-NHS (10 equiv relative to the amount of oligodeoxynucleotide on ODN approximately) for 24 h. The solid-phase CPG was washed with DMF (3×500 μL) and acetonitrile (3×500 μL). After deprotection and HPLC purification, the desired TAMRA conjugated ODN was obtained in 44% yield according to HPLC trace at 260 nm. MALDI-TOF: *m/z*: calcd: 5345; found: 5345.

Attachment ODN 3 with pyrenemethylamine: **ODN 3** on CPG was suspended in acetonitrile/water (v/v 4/1, 6 mg mL^{-1}) in a quartz cell. After 365 nm UV irradiation for 30 min, the CPG was washed with acetonitrile and dried. The residue was suspended in a DMSO solution (100 μL) of BOP, HOBt, DIPEA and pyrenemethylamine hydrochloride (10 equiv). After having been shaken for 24 h at room temperature, the solid phase was washed with DMF (3×500 μL), acetonitrile (3×500 μL) and ether (500 μL). After hydrolysis and HPLC purification, the desired ODN conjugated with pyrene was obtained in 82% yield. MALDI-TOF *m/z*: calcd: 5162; found: 5163.

Attachment ODN 8 with 1-pyrenebutyric acid: **ODN 8** on CPG was suspended in acetonitrile/water (v/v 4/1, 6 mg mL^{-1}) in a quartz cell. After 365 nm UV irradiation for 30 min, the solution was evaporated to dryness. The solution of BOP, HOBt, DIPEA and 1-pyrenebutyric acid in DMF (100 μL) was added to the dry power and the mixture was then shaken for 24 h at room temperature. After evaporation, hydrolysis and desalting, the HPLC trace showed 85% of labelling yield. After detritylation, MALDI-TOF confirmed the expected ODN: calcd: 4872; found: 4874.

Cyclisation of ODN 11: **ODN 11** on CPG was suspended in acetonitrile/water (v/v 4/1, 6 mg mL^{-1}) in a quartz cell. After 365 nm UV irradiation for 30 min, the solution was evaporated to dryness. The solution of BOP (or HBTU/EDC·HCl for the comparison), HOBt and DIPEA in DMF (100 μL) was added to the dry power,. The mixture was shaken for 24 h at room temperature. After evaporation, hydrolysis, desalting and HPLC purification, MALDI-TOF confirmed the desired fractions: linear: calcd: 4796; found: 4799; circular: calcd: 4779; found: 4783.

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