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# New light labile linker for solid phase synthesis of 2′-*O*-acetalester oligonucleotides and applications to siRNA prodrug development

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ABSTRACT

We report on the synthesis and properties of oligonucleotides containing 2′-*O*-(levulinic acid) and 2′-*O*-(amino acid) acetalesters. Given that esters serve as promoieties in several therapeutic prodrugs, we believe that these derivatives will have potential use as nucleic acid prodrugs. In addition, we report on the synthesis of a novel solid support with a photolabile linker that not only allows for the synthesis of oligonucleotides containing various 2′-*O*-acetalesters, but can be generally adopted to the synthesis of base-sensitive oligoribonucleotides. The release of oligonucleotides from this support is faster than with conventional linkers.



Harnessing RNA interference (RNAi)[1] for therapeutic gene silencing presents an attractive alternative to small molecule approaches. Unfortunately, *in vivo* gene silencing in a therapeutic setting has proven difficult. The major obstacle of RNAi based therapeutics is the cellular delivery of siRNA since these are generally too large (~13 kDa), too negatively charged (~40 negative charges) and not hydrophobic enough for efficient intracellular uptake.[2] Rapid nuclease degradation and clearance reduces the in vivo duration of activity of siRNAs, and nonspecific immune responses pose challenges for siRNA therapeutics. To overcome these problems, research has mostly focused on the use of delivery vehicles such as liposomes, lipids, cationic polymer complexes or lipophilic conjugates,[3] while chemically modified siRNAs have been used to increase both serum and cellular stability and to prevent the activation of the immune response.[4]

Another approach that may be used to overcome the difficulties experienced in delivery would be the use of siRNA prodrugs. Prodrugs are bioreversible derivatives of drug molecules that undergo an enzymatic and/or chemical transformation in vivo to release the active parent drug, which can then exert the desired pharmacological effect.[5]

Though the prodrug approach has been widely successful for small molecules, this strategy has been sparingly used for oligonucleotide therapeutics,[6] and there are no reports for its use in siRNA silencing experiments. Nevertheless, there are several positions on an oligoribonucleotide chain that may be amenable to conjugation with a promoiety.

Modification of the internucleotide phosphate linkage has received the most attention in terms of a prodrug approach due to its negative charge. Examples involve the *S*-acylthioethyl,[7] the *S*-acyloxymethyl[8] and the *O*-(4-acyloxybenzyl)-phosphate(thioate)[9] moieties. The 5-nitro-2-furylmethyl and 5-nitro-2-thienylmethyl groups have also shown to increase cell permeability. These groups are activated by an endogenous nitroreductase, which in turn starts a cascade reaction releasing the active drug molecule.[10] Another very attractive modification, the 2-(*N*-formyl-*N*-methyl)-aminoethyl thiophosphate protecting group, is cleaved by the temperature of the cellular environment (37 °C).[11] Its promoiety ability was tested in immunomodulatory CpG oligonucleotides that effectively protected mice from viral infections.[11]

Other potential sites for modification, the 5′- and 3′-hydroxyl positions at the terminal ends of an oligonucleotide, have been extensively used for covalent attachment of a variety of groups to aid in cellular uptake, for example, cholesterol and lauric acid,[12] but because there are only two positions available for modification, it has received no attention as a prodrug approach.

Finally, the ribose 2′-hydroxyl position has traditionally been a very popular position for chemical modification on RNA chains, particularly to enhance hybridization affinity, lipophilicity and nuclease stability.[13] Surprisingly, it has received little attention for the prodrug strategy.

We have previously reported on the acetal levulinic ester (ALE) as a promising protecting group for the in situ synthesis of RNA microarrays.[14] Given that esters are involved in several therapeutic prodrugs, we expected acetalesters to be a promising prodrug moiety. To the best of our knowledge, the 2′-*O*-acetalester of Debart and co-workers is the only system disclosed thus far.[15,16] The authors used acetyl, propyl, butyl, *iso*-butyl and pivaloyl acetalesters in a preliminary study of proRNA. They used the Q-linker together with a polystrene (PS) solid support and released the oligonucleotides with 3:1 $NEt_3$/48% HF (aq). Notably, the bulkier pivaloyl acetalester was the only one that remained intact under these conditions. With this in mind we decided to investigate the 2′-*O*-ALE and various 2′-*O*-(amino acid) acetalesters as prodrug

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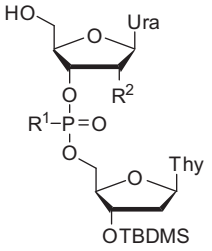
moieties. We hypothesized that the resulting proRNA molecules, especially the zwiterionic versions (2′-*O*-amino acids) should have enhanced binding affinity and cellular uptake by virtue of the positively charged amino groups. Furthermore, with 20 amino acids to choose from, it should allow for tuning of the physicochemical properties and delivery of these RNAs.

A 15-nt long DNA-RNA chimeric strand was prepared (5′-$dt_9$-$U_{ALE}$-$dt_5$-3′) on a Q-linker PS solid support.[17] Polystyrene was chosen as the solid support since it is stable to the final fluoride treatment required to release the oligonucleotide into solution. The oligonucleotide was synthesized using both commercially available thymidine phosphoramidite and our previously disclosed uridine 2′-*O*-ALE phosphoramidite, both of which were activated using 5-(ethylthio)-tetrazole (ETT).[14] The thymidines were installed using standard conditions and reaction times while the 2′-*O*-ALE uridine monomer was coupled for 15 min at room temperature. Standard capping, oxidation and detritylation steps followed the coupling steps. After the completion of the synthesis, the solid support was treated with 2:3 $NEt_3$/MeCN for 16 h to remove β-cyanoethyl protecting groups. The material was then treated with 3:1 $NEt_3$/48% HF (aq) for 8 h to release the oligonucleotide from the polystyrene support. The crude material was analyzed by HPLC and yielded two major peaks in an approximately 2:1 ratio (SI). The first eluting peak had the same retention time as an authentic, fully deprotected oligonucleotide. The major peak eluted ~2 min later, suggesting that this compound was the desired 2′-*O*-ALE modified strand. MS analysis confirmed the fully deprotected and the more retained ALE-modified strand (entry 1, Table 1). The ALE-modified compound was isolated, lyophilized, redissolved in water and re-injected into the HPLC and the material eluted again as two peaks, suggesting that the ALE group undergoes slow cleavage under the HPLC purification conditions.

To evaluate the decyanoethylation conditions required for 2′-*O*-acetalester containing oligonucleotides, a 2′-*O*-ALE containing dimer (**1a**, Fig. 1) was synthesized as a model system and the stability of the dimer was examined by $^{31}$P-NMR under the various conditions. First, compound **1a** was treated with 2:3 $NEt_3$/MeCN at room temperature. To our surprise, under these conditions the time needed for decyanoethylation was over 24 h. However, when the mixture was heated to 50 °C under these conditions, the time for complete decyanoethylation was reduced to one hour with no trace of ALE cleavage (SI), demonstrating the stability of the ALE group during deprotection of the phosphate group.

In order to test the stability of the 2′-*O*-acetalester group to HPLC purification (0.1 M aq TEAA buffer), the model compound **1b**, was first dissolved in $D_2O$ and monitored by $^{31}$P-NMR over a 7-day period. After 1 day there was approximately 20% cleavage of the ALE-group, with no subsequent decomposition observed after 7 days. The dimer was also dissolved in 0.1 M TEAA in $D_2O$, pD 7.2 and monitored in the same manner as above over 7 days. Again, after 1 day there was approximately 20% cleavage of the ALE-group and this increased to 30% after 7 days. These results indicate that while the ALE group is fairly stable in $D_2O$ and under the buffer conditions used during HPLC purification (SI), extended manipulation in water results in mixtures of compounds.




**1** a: $R^1 = O(CH_2)_2CN$, $R^2 = OCH_2C(O)(CH_2)_2C(O)CH_3$
b: $R^1 = O^{\ominus}$, $R^2 = OCH_2C(O)(CH_2)_2C(O)CH_3$
c: $R^1 = O^{\ominus}$, $R^2 = OH$

**Figure 1.** Dimer used in the study of the stability of 2′-*O*-ALE group.

We therefore turned our attention to photocleavable linkers, which in principle can be cleaved to release the intact acetalester modified RNA. An added advantage of this approach is that the oligonucleotide can be deprotected while it is attached to the solid support and be released quickly into anhydrous MeCN, minimizing extended aqueous deprotection steps and avoiding hazardous HF solutions. Previously reported photocleavable supports include those based on an *o*-nitrophenyl-1,3-propanediol linker,[18] as well as **2** (Fig. 2), designed by Venkatesan and Greenberg.[19] The latter linker, however, requires 1–2 h of irradiation in MeCN/$H_2O$ to obtain a satisfactory level of cleavage from oligonucleotides. It was hypothesized that extending the chain by one carbon atom, and branching it to generate a tertiary carbon centre, the linker would resemble the photolabile NPPOC-protecting group[20] (**3**, Fig. 2) and, hence, it was anticipated to be cleaved in a shorter period of time relative to **2**. The linker, orthogonal to esters and acetalesters, was conjugated to the nucleoside not through a traditional carbonate to the 3′-end, but through an internucleotide phosphate. In this way, the use of phosgene, which is highly toxic, or other phosgene alternatives, which are difficult to remove, can be avoided. The linker was prepared according to Scheme 1, by first reacting compound **5**[21] with FmocCl in pyridine to generate **6** in 95% yield. This was followed by treatment with 80% TFA in $CH_2Cl_2$ to liberate the free acid **7**, which then was ready to couple to the solid support. Compound **7** was conjugated to aminomethyl polystyrene (stratosphere for DNA synthesis, 1000 Å) or long chain alkyl amine controlled pore glass (LCAA-CPG, 500 Å) using HATU and DMAP in pyridine, followed by the capping of unreacted amines with CAP A and CAP B solutions ($Ac_2O$/Pyridine/*N*-methyl-imidazole/THF) to give **8**. The Fmoc was removed with 10% 4-methylpiperidine in DMF to give **9**. Compounds **10**[22] and **11** were stirred at

**Table 1**
MS of oligonucleotides

| Entry | Target sequence and hydrolysis products | Mass calculated | Mass found |
|---|---|---|---|
| 1[a] | 5′-$dt_9$-U-$_{ALE}$-$dt_5$-3′ | 4834.27 | 4834.42 |
| | 5′-$dt_9$-U-$dt_5$-3′ | 4705.21 | 4706.19 |
| 2[b] | 5′-$dt_9$-$U_{APhe}$-$dt_5$-3′-lv | 4778.0 | 4778.3 |
| | 5′-$dt_9$-U-$dt_5$-3′-lv | 4600.9 | 4601.1 |
| | 5′-$dt_9$-U-$dt_5$-3′ | 4502.9 | 4503.2 |
| 3[b,c] | 5′-$dt_5$-$U_{APhe}$-$dt_4$-$U_{APhe}$-$dt_4$-$U_{APhe}$-$dt_5$-3′-lv | 6961.4 | 6963 |
| | Two $U_{APhe}$ inserts | 6784.3 | 6783 |
| | One $U_{APhe}$ insert | 6607.2 | 6606 |

[a] Q-linker used with PS solid support. MALDI-TOF results include spermine.
[b] Light labile linker used with CPG solid support. ESI–MS results.
[c] MS done on crude sample.

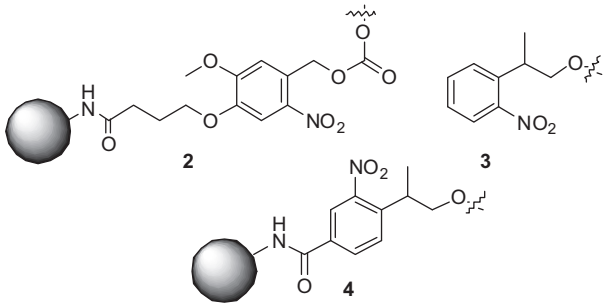
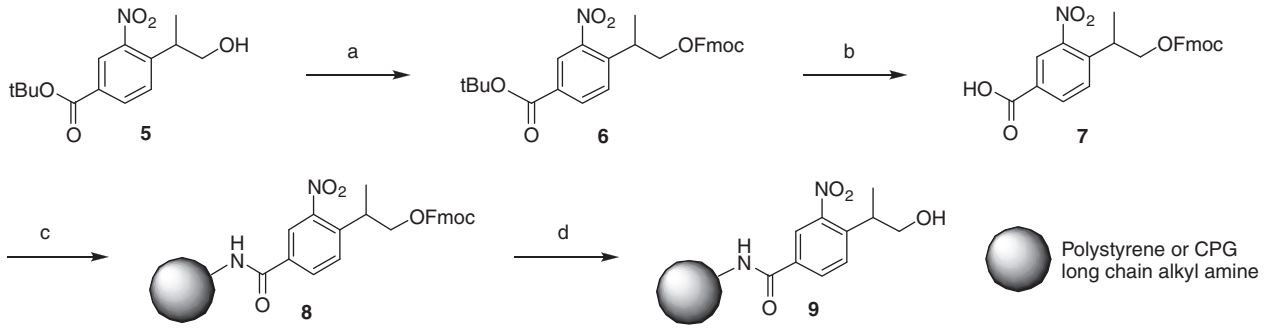
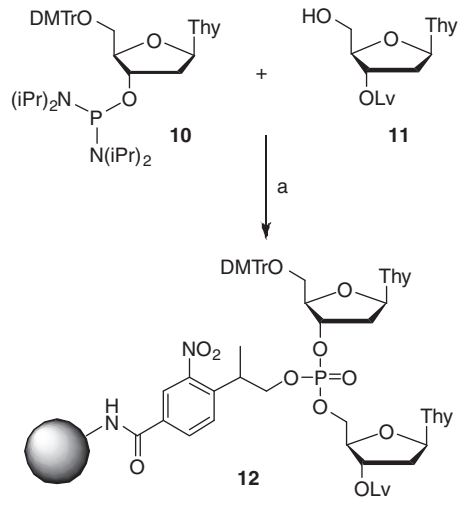



**Figure 2.** Structure of the solid support developed by Greenberg and co-workers (**2**) of the NPPOC-protectiong group (**3**) and of the solid support developed in this work (**4**).




**Scheme 1.** Reagent and conditions: (a) Fmoc-Cl, pyridine, rt, 2 h, 95%; (b) 80% TFA in $CH_2Cl_2$, rt. 30 min, 93%; (c) Aminomethylated polystyrene or CPG, HATU, DMAP, Pyridine, Capping ($Ac_2O$, *N*-Methyl imidazole, pyridine, THF), rt, 18 h; (d) 4-methyl piperidine, DMF, rt, 1.5 h.




**Scheme 2.** Reagents and conditions: (a) (i) DCI in MeCN, rt, 7 h, (ii) 5, rt, 18 h, (iii) oxidation ($I_2$/$H_2O$/pyridine/THF), rt, 0.5 h, (iv) capping ($Ac_2O$, *N*-Methyl imidazole, pyridine, THF), rt, 1.5 h. Loading 35–60 μmol/g

room temperature with 0.5 M DCI in MeCN for 7 h and the resultant mixture was then added to **9** (Scheme 2). The phosphite triester intermediate was then oxidized ($I_2$/$H_2O$/pyridine/THF) and the unreacted free hydroxyl groups were capped with $Ac_2O$/Pyridine/*N*-methyl-imidazole/THF. The support was obtained with a loading of 35–60 μmol/g of support, as assessed by the trityl color assay.[23]

The next step to test was the cleavage of an oligonucleotide from the solid support, thus a 21-nt chimeric DNA/RNA strand (5′-$dt_5$-U-$dt_4$-U-$dt_4$-U-$dt_5$-3′-Lv) was synthesized. After cleavage of the β-cyanoethyl protecting groups with anhydrous 2:3 $NEt_3$/MeCN, overnight and then at 65 °C for 1 h, the material was dissolved in anhydrous 1% DIPEA in MeCN and subjected to photolysis in a transilluminator with an irradiation source in the UV-A region.[24]

The cleavage was followed over time (Fig. 3A). A 15-nt uridine sequence was also synthesized on a 1 μmol scale, deprotected as above, and photolyzed at room temperature for 20 min. The oligonucleotide was washed off the support and dried, followed by de-silylation with 1.5:0.75:1 $NEt_3$-3HF/NMP/$NEt_3$ for 4 h, 65 °C, yielding 44O.D.s (0.31 μmol) of the crude oligonucleotide. The recovered crude material eluted as one peak (HPLC; 92% purity; Fig. 3B) that was confirmed to be the correct oligonucleotide by MS (Calcd 4526.6; Found 4526.6).

With a fast-working solid support that does not require aqueous basic cleavage conditions, our attention turned to amino acid based acetalesters (AAE). Examination began with alanine, due to its simplicity; lysine, due to its increased net positive charge; and phenylalanine, due to its hydrophobicity and increased size. The synthesis of the monomers is described in Scheme 3. Compound **13**[25] was activated with sulfuryl chloride and then reacted with the cesium salt of the desired Fmoc-protected amino acid to obtain **14a**–**c** in 78–90% yield. The bis-silyl protecting group was cleaved using $NEt_3$·3HF in MeCN to give **15a**–**c** in 61–98% yield. Compound **15a**–**c** was tritylated under standard conditions to give **16a**–**c** in 66–78% yield, followed by phosphitylation to give the final phosphoramidites **17a**–**c** in 58–86% yield.

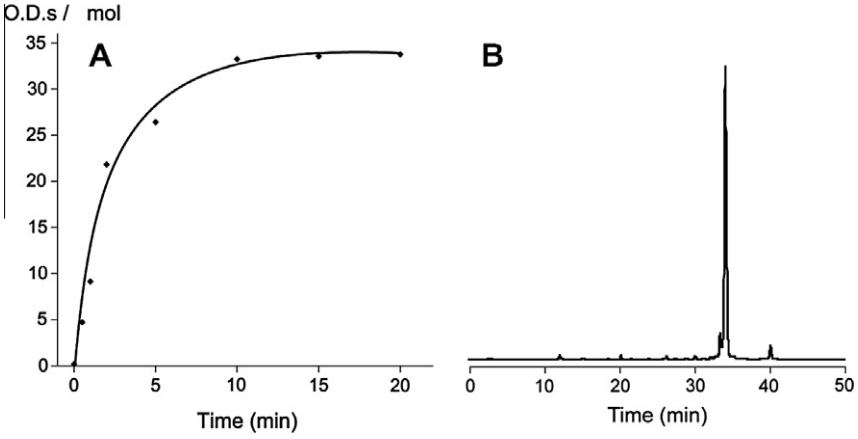
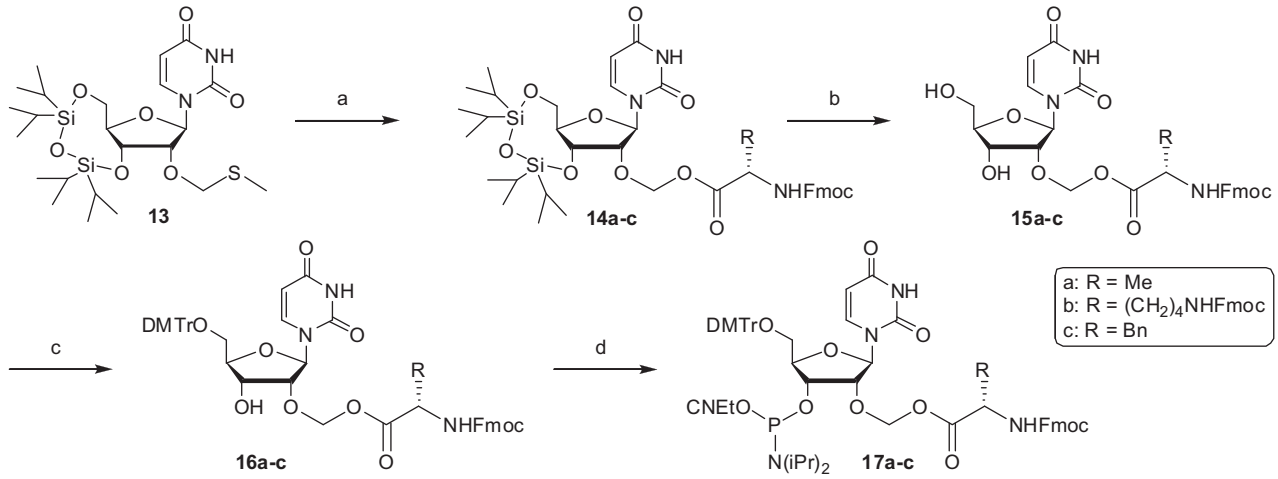



**Figure 3.** (A) Rate of oligonucleotide (5′-$dt_5$-U-$dt_4$-U-$dt_4$-U-$dt_5$-3′-Lv) release when solid support **12** is placed in MeCN with 1% DIPEA under a transilluminator. (B) Crude HPLC of poly uridine synthesized with solid support **12**.




**Scheme 3.** Reagent and conditions: (a) (i) $SO_2Cl_2$, $CH_2Cl_2$, 0 °C–rt, 2 h, (ii) Cs-Amino acid-Fmoc, DMF, $CH_2Cl_2$, rt, 18 h, **14a** 90%, **14b** 88%, **14c** 78%; (b) $NEt_3{\cdot}3HF$, MeCN, rt, 18 h, **15a** 87%, **15b** 98%, **15c** 61%; (c) DMTrCl, $AgNO_3$, Pyridine, THF, rt, 2.5 h, **16a** 76%, **16b** 66%, **16c** 78%; (d) $CNEtOP(Cl)N(iPr)_2$, DIPEA, THF, rt, 5.5 h, **17a** 86%, **17b** 58%, **17c** 62%.

With the amidites in hand, 15-nt chimeras with single acetal amino ester (AAE) inserts (5′-$dt_9$-$U_{AAE}$-$dt_5$-3′-Lv, $U_{AAE}$ = Ala **18a**; Lys **18b**; Phe **18c**) were synthesized and deprotected on a 1 μmol scale using the same conditions as described above followed by RP–HPLC analysis. For the Ala- and Lys-containing oligonucleotides (**18a** and **18b**), two peaks were observed in the chromatogram, one major peak corresponding to 5′-$dt_9$-U-$dt_5$-3′-Lv and a minor, less retained peak corresponding to the fully deprotected oligonucleotide (5′-$dt_9$-U-$dt_5$-3′). Therefore, the desired Ala and Lys-containing oligonuclesotide could not be obtained. For the Phe-containing oligonucleotide (**18c**), three peaks were observed by HPLC: 5′-$dt_9$-U-$dt_5$-3′ and 5′-$dt_9$-U-$dt_5$-3′-Lv, as observed previously, and a more retained, prominent peak corresponding to the desired Phe-containing oligonucleotide, 5′-$dt_9$-$U_{APhe}$-$dt_5$-3′-Lv (peak 3; Fig. 4A). The three oligonucleotides were confirmed by MS (entry 2, Table 1). The compound was isolated, lyophilized and re-injected into the HPLC, and the material eluted again as three peaks, indicating that the Phe-acetalester also hydrolyzed (albeit more slowly relative to Ala and Lys) during HPLC purification/handling (Fig. 4B).

To further examine the properties of the Phe-containing oligonucleotide, a three-insert sequence was prepared (5′-$dt_5$-$U_{APhe}$-$dt_4$-$U_{APhe}$-$dt_4$-$U_{APhe}$-$dt_5$-3′-Lv, **19**).

Five distinguishable peaks were observed by HPLC, among which were the desired product and two others with one and two $U_{APhe}$ inserts (MS on the crude sample; entry 3, Table 1). The free oligonucleotide was not detected by MS. As anticipated, the desired product could not be isolated in pure form after attempted separation by HPLC. Incubation of the crude mixture in phosphate buffer (pH 7.2) at 37 °C showed that the peak corresponding to 5′-$dt_5$-$U_{APhe}$-$dt_4$-$U_{APhe}$-$dt_4$-$U_{APhe}$-$dt_5$-3′-Lv disappeared within 5 h, with complete conversion of **19** to 5′-$dt_5$-U-$dt_4$-U-$dt_4$-U-$dt_5$-3′-Lv occurring within 48 h (SI).

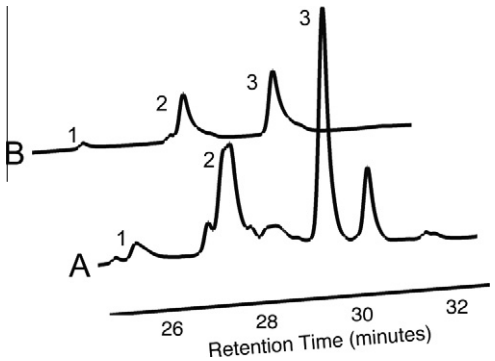



**Figure 4.** Peak 1: 5′-$dt_9$-U-$dt_5$-3′, Peak 2: 5′-$dt_9$-U-$dt_5$-3′-Lv, Peak 3: 5′-$dt_9$-$U_{APhe}$-$dt_5$-3′-Lv, **18c**. (A) Crude RP-HPLC of 15-nt with one phenylalanine insert. (B) Mixture obtained after re-injection of peak 3 isolated from mixture shown in trace A.

In summary, we have developed and synthesized a new photolabile linker for the solid-supported synthesis of oligoribonucleotides. The photolabile linker is cleaved within 15 min at room temperature under anhydrous conditions. With the newly developed solid support, various amino acid conjugated oligonucleotides were synthesized. Different cleavage rates were seen among various amino acids tested, in which phenylalanine was cleaved at the slowest rate. The ease of cleavage of the phenylalanine acetalester from the parent RNA in the absence of esterases warrant further studies with amino acid derivatives of siRNAs. Cell uptake studies are ongoing in our laboratory.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.04.073.

## References and notes

1. (a) Fire, A.; Xu, S.; Montgomery, M. K.; Kostas, S. A.; Driver, S. E.; Mello, C. C. *Nature (London, U. K.)* **1998**, *391*, 806; (b) Zimmermann, T. et al *Nature (London, U. K.)* **2006**, *441*, 111.
2. Whitehead, K. A.; Langer, R.; Anderson, D. G. *Nat. Rev. Drug Discovery* **2009**, *8*, 129.
3. Aagaard, L.; Rossi, J. J. *Adv. Drug Deliv. Rev.* **2007**, *59*, 75.
4. Watts, J. K.; Deleavey, G. F.; Damha, M. J. *Drug Discovery Today* **2008**, *13*, 842.
5. (a) Caldwell, H. C.; Adams, H. J.; Rivard, D. E.; Swintosky, J. V. *J. Pharm. Sci.* **1967**, *56*, 920; (b) Stella, V. J. In *Prodrugs: Challenges and Rewards*; Stella, V. J., Oliyai, R., Borchardt, R. T., Maag, H., Hageman, M. J., Tilley, J. W., Eds.; Springer: New York, 2007; pp 3–33; (c) Rautio, J.; Kumpulainen, H.; Heimbach, T.; Oliyai, R.; Oh, D.; Jarvinen, T.; Savolainen, J. *Nat. Rev. Drug Discovery* **2008**, *7*, 255.
6. Poijärvi-Vitra, P.; Lönnberg, H. *Curr. Med. Chem.* **2006**, *13*, 3441.
7. (a) Tosquellas, G.; Bologna, J. C.; Morvan, F.; Rayner, B.; Imbach, J.-L. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2913; (b) Tosquellas, G.; Alvarez, K.; Dell'Aquila, C.; Morvan, F.; Vasseur, J. J.; Imbach, J. L.; Rayner, B. *Nucleic Acids Res.* **1998**, *26*,

2069; (c) Spinelli, N.; Vasseur, J. J.; Hayakawa, Y.; Imbach, J. L. *Nucleosides, Nucleotides, Nucleic Acids* **2001**, *20*, 947.
8. Iyer, R. P.; Yu, D.; Agrawal, S. *Bioorg. Chem.* **1995**, *23*, 1.
9. Iyer, R. P.; Ho, N.-h.; Yu, D.; Agrawal, S. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 871.
10. Zhang, N.; Tan, C.; Cai, P.; Zhang, P.; Zhao, Y.; Jiang, Y. *Chem. Commun. (Cambridge, U. K.)* **2009**, 3216.
11. Grajkowski, A.; Pedras-Vasconcelos, J.; Wang, V.; Ausin, C.; Hess, S.; Verthelyi, D.; Beaucage, S. L. *Nucleic Acids Res.* **2005**, *33*, 3550.
12. (a) Lorenz, C.; Hadwiger, P.; John, M.; Vornlocher, H.-P.; Unverzagt, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4975; (b) Shoutschek, J. et al *Nature (London U. K.)* **2004**, *432*, 173.
13. (a) Manoharan, M. *Biochim. Biophys. Acta Gene Regul. Mech.* **1999**, *1489*, 117; (b) Mangos, M. M.; Damha, M. J. *Curr. Top. Med. Chem. (Hilversum, Neth.)* **2002**, *2*, 1147.
14. Lackey, J. G.; Mitra, D.; Somoza, M. M.; Cerrina, F.; Damha, M. J. *J. Am. Chem. Soc.* **2009**, *131*, 8496.
15. (a) Parey, N.; Baraguey, C.; Vasseur, J.-J.; Debart, F. *Org. Lett.* **2006**, *8*, 3869; (b) Martin, A. R.; Lavergne, T.; Vasseur, J.-J.; Debart, F. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4046.
16. Lavergne, T.; Parey, N.; Vasseur, J.-J.; Debart, F. *Eur. J. Org. Chem.* **2009**, 2190.
17. Pon, R. T.; Yu, S. *Nucleic Acids Res.* **1997**, *25*, 3629.
18. Dell'Aquila, C.; Imbach, J.-L.; Rayner, B. *Tetrahedron Lett.* **1997**, *38*, 5289.
19. Vankatesan, H.; Greenberg, M. M. *J. Org. Chem.* **1996**, *61*, 525.
20. Hasan, A.; Stengele, K.-P.; Giegrich, H.; Cornwell, P.; Isham, K. R.; Sachleben, R. A.; Pfleiderer, W.; Foote, R. S. *Tetrahedron* **1997**, *53*, 4247.
21. Smirnova, J.; Wöll, D.; Pfeiderer, W.; Steiner, U. E. *Helv. Chim. Acta* **2005**, *88*, 891.
22. Grajkowski, A.; Cieslak, J.; Kauffman, J. S.; Duff, R. J.; Norris, S.; Freedberg, D. I.; Beaucage, S. L. *Bioconjug. Chem.* **2008**, *19*, 1696.
23. Pon, R. T. In *Current Protocols in Nucleic Acid Chemistry*; Egli, M., Herdewijn, P., Matusda, A., Sanghvi, Y., Eds.; Wiley: Hoboken, 2000; pp 3.2.12–3.2.13. Unit 3.2.
24. Photoreactor Luzchem, Hitachi FL8BL-B bulbs, $\lambda_{max}$ = 350 nm
25. (a) Zavgorodny, S.; Polianski, M.; Besidsky, E.; Kriukov, V.; Sanin, A.; Pokrovakaya, M.; Gurskaya, G.; Lönnberg, H.; Azhayev, A. *Tetrahedron Lett.* **1991**, *32*, 7593; (b) Rastogi, H.; Usher, D. A. *Nucleic Acids Res.* **1995**, *23*, 4872.