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Published on Web 02/08/2003

A Conformationally Preorganized Universal Solid Support for Efficient Oligonucleotide Synthesis

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The automated synthesis of oligonucleotides is often carried out on solid supports containing 3′-terminal nucleosides attached via a readily cleavable ester linkage. One limitation of this approach is that a minimum of four solid supports is required for the preparation of unmodified oligodeoxynucleotides. For the synthesis of novel oligonucleotides, a continuously growing number of supports carrying other modified nucleosides and 3′-terminal modifiers are required. In a more straightforward approach, a 3′-terminal nucleoside 3′-*O*-phosphoramidite is coupled to the hydroxy group of a universal linker, a support-bound mono-acylated 1,2-diol, via a 3′-*O*-phosphodiester or -triester moiety.[1−3] Exposure of an assembled oligonucleotide to aqueous ammonium hydroxide or other bases removes the acyl protection. The released hydroxy group transesterifies the phosphate moiety so that the oligonucleotide is dephosphorylated at the 3′-terminus and a derivative of ethylene phosphate is formed as a side product. This approach eliminates the need for preparation of sequence-specific solid supports.

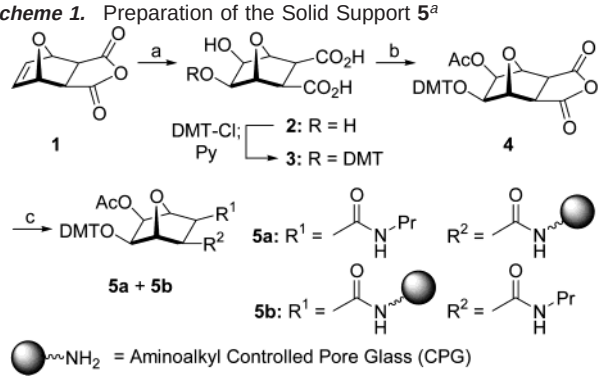
The common limitation of the reported universal linkers is that the release of oligonucleotides requires prolonged heating with concentrated ammonium hydroxide[1,2] or it occurs under orthogonal conditions.[3a] Moreover, because their structure−activity relationship has not been extensively studied, further improvement of these reagents has been retarded.

In the hydrolysis of RNA, one of the key factors governing the reaction rate is the distance between the 2′-O and P(V) atoms.[4] We hypothesized that locking the two vicinal C−O bonds of an alkyl 2-hydroxyethyl phosphate in a *syn*-periplanar conformation might reduce the distance between the 2-O and P(V) atoms and hence increase the rate of phosphodiester hydrolysis.

To test the hypothesis, a solid support **5** that featured two protected hydroxy groups in the vicinal positions with the required *syn*-periplanar orientation was synthesized as depicted in Scheme 1 and tested in the oligonucleotide synthesis (Scheme 2). The protected 20-mer phosphodiester (PO) oligonucleotides **11**−**14**, their phosphorothioate (PS) analogues **15**−**18**, and chimeric oligonucleotides **19**−**25** (Scheme 2 and Table 1) were assembled on the solid support **5** using the phosphoramidite building blocks **28**−**30** (Chart 1).

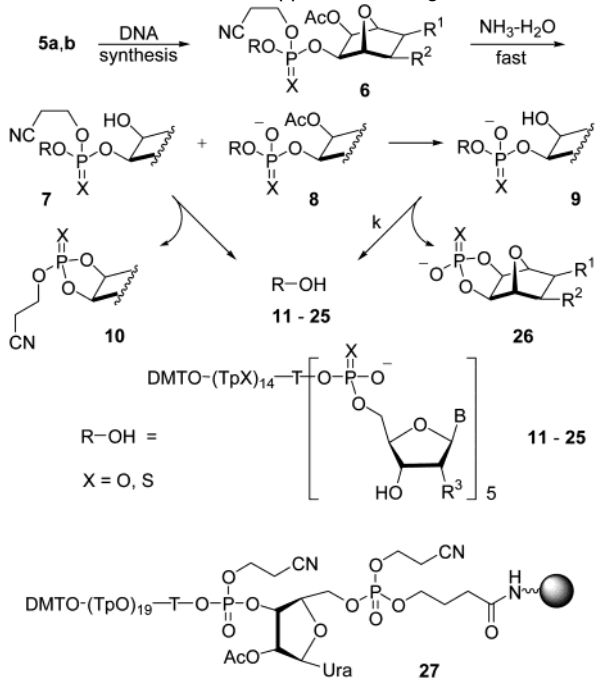
The support-bound oligonucleotides **6** (Scheme 2) were treated with concentrated aqueous ammonium hydroxide for 6 h at room temperature, and the liquid phase was then heated for 8 h at 55 °C. The product distribution in the deprotection mixtures was analyzed by reverse-phase HPLC and ES MS to prove that the intended compounds **11**−**25** (Table 1) were indeed present in the mixtures as the predominant products. It is important to note that neither the corresponding oligonucleotide-3′-phosphates nor any other 3′-modified products could be detected in the reaction mixtures. Comparison of the crude yields of **11**−**18** and **21**−**24** with those obtained by synthesis on conventional 3′-*O*-succinyl nucleoside solid supports did not reveal any significant differences.

Scheme 1. Preparation of the Solid Support **5**[a]



[a] (a) $OsO_4/H_2O_2/H_2O$/acetone/ether/*t*BuOH; (b) Ac_2O/pyridine; (c) (1) aminoalkyl CPG/Py, (2) Ac_2O/NMI/Py, (3) HATU/HOBT/MeCN/Py then *n*$PrNH_2$/MeCN, (4) Ac_2O/NMI/Py.

Scheme 2. Synthesis of Oligonucleotides **11**−**25** on Solid Support **5** and the Structure of Solid Support-bound Oligonucleotide **27**



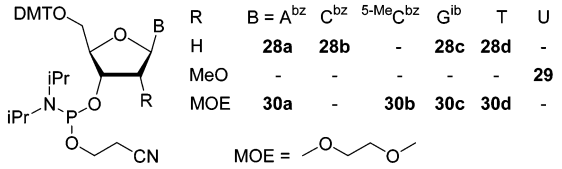
The kinetics of the release of the oligonucleotides **11**−**25** from the solid support **6** was next studied by the continuous flow method[5] with appropriate modifications.[6] Essentially, concentrated aqueous ammonium hydroxide (14.3 M, 27.1%) was passed through the solid support **6** at a constant flow rate and at a temperature of 300.15 K, and the UV absorbance of the eluate containing the released **11**−**25** was recorded as a function of time. The data obtained for the release of **14** are shown in Figure 1.

10.1021/ja0284613 CCC: $25.00

Table 1. Time Required for 95% Release of Oligonucleotides **11**−**25** from the Universal Solid Support **6** with 14.3 M Aqueous Ammonium Hydroxide at 300.15 K

oligonucleotide				
compound	B	R^3	X	95% release, min
11	A	H	O	238
12	G	H	O	272
13	C	H	O	251
14	T	H	O	256
15	A	H	S	343
16	G	H	S	456
17	C	H	S	365
18	T	H	S	382
19	G	MOE	O	180
20	5-Me-U	MOE	O	129
21	A	MOE	S	161
22	G	MOE	S	212
23	5-Me-C	MOE	S	159
24	5-Me-U	MOE	S	182
25	U	OMe	O	123

Chart 1. Phosphoramidite Building Blocks **28**−**30**



R	B = A^{bz}	C^{bz}	$^{5\text{-Me}}C^{bz}$	G^{ib}	T	U
H	**28a**	**28b**	-	**28c**	**28d**	-
MeO	-	-	-	-	-	**29**
MOE	**30a**	-	**30b**	**30c**	**30d**	-

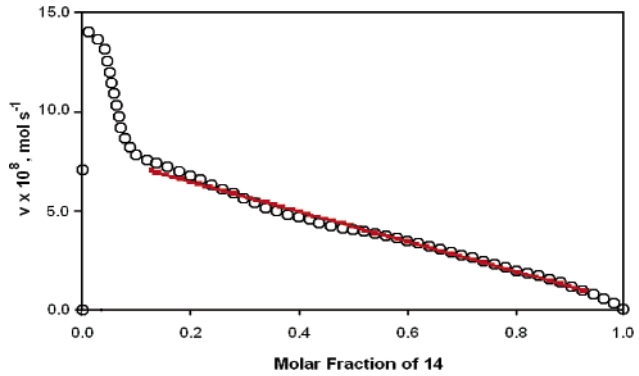


Figure 1. The rate of the release of **14** from **6** (○) as a function of the extent of the release of **14** (14.3 M aqueous ammonium hydroxide, 300.15 K). The linear fit is shown as a red line.

As suggested in Scheme 2, the solid support **6** may rapidly undergo two concurrent reactions to give the intermediates **7** and **8**. The phosphotriester **7** may rapidly release the oligonucleotides **11**−**25** or it may form **9** concurrently. Compound **8** can only be converted to **9**, which then releases **11**−**25** to the solution at a slower rate. Indeed, the plot in Figure 1 demonstrates that **14** was released via two concurrent processes. A faster step, which may be attributed to the release of **14** (ca. 15%) via hydrolysis of **7**, was virtually complete in less than 20 min, while the majority of oligonucleotide material on the solid phase was believed to be accumulated in **9**. The release of **14** via hydrolysis of **9** obeyed the kinetic law for a pseudo-first-order reaction in solution with the apparent rate constant k of 16.8×10^{-5} s^{-1}. By comparison, **14** was released from the reported **27**[1c] (Scheme 2) with the rate constant $k = 1.3 \times 10^{-5}$ s^{-1} (12.9 times more slowly), which strongly supported the positive effect of conformational preorganization in **9**.[6]

Because of the high complexity of the kinetic scheme, it was not possible to extract all of the rate constants involved. To provide guidelines for the practical applications of **5** in oligonucleotide synthesis, the time required for 95% release of the oligonucleotides **11**−**25** from **6** was measured (Table 1).

Comparison of these release times demonstrated that compounds **19**−**25** bearing 2′-*O*-alkylribonucleoside residues at the 3′-terminus were released 1.5−2.3 times faster than the corresponding 2′-deoxy counterparts **12** and **14**−**18**. The release of **20** was comparable to that of **25**, which suggested that the rate acceleration could be attributed to the presence of the 2′-oxygen rather than to the structure of the 2′-*O*-substituent. One might argue that the introduction of the 2′-alkoxy group to the 3′-terminal nucleoside residue decreased the pK_a value of the 3′-hydroxy group, which facilitated the departure of the 3′-terminal nucleoside.

Throughout the series, a positive "thio-effect" was observed; that is, PO oligonucleotides were released 1.2−1.7 times faster than the corresponding PS analogues. No substantial dependence of the release time on the structure of the 3′-terminal base moiety was observed. However, compounds **12**, **16**, **19**, and **22** where B = G were released 6−39% more slowly than the corresponding compounds with other bases, and, thus, for each type of backbone and sugar modification studied, these oligonucleotides represented the worst case scenario.

In conclusion, the universal support **5** is fully compatible with the conditions of oligonucleotide synthesis. On treatment with aqueous ammonium hydroxide, the oligonucleotides are quantitatively released with favorable kinetics.

Supporting Information Available: Experimental procedures for **2**−**5** and **27**, spectral data for **2**−**4**, HPLC profiles and ES mass spectra for **11**−**25** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Universal solid supports derived from uridine: (a) Crea, R.; Horn, T. *Nucleic Acids Res.* **1980**, *8*, 2331−2348. (b) Schwartz, M. E.; Breaker, R. R.; Asteriadis, G. T.; Gough, G. R. *Tetrahedron Lett.* **1995**, *36*, 27−30. (c) Lyttle, M. H.; Dick, D. J.; Hudson, D.; Cook, R. M. *Nucleosides Nucleotides* **1999**, *18*, 1809−1824.

(2) Universal supports derived from *cis*-3,4-dihydroxytetrahydrofuran: (a) Scott, S.; Hardy, P.; Sheppard, R. C.; McLean, M. J. In *Innovation Perspect. Solid-Phase Synthesis*; Epton, R., Ed.; Mayflower Worldwide: Birmingham, U.K., 1994; pp 115−124. (b) Scheuer-Larson, C.; Rosenbohm, C.; Jorgensen, T. J. D.; Wengel, J. *Nucleosides Nucleotides* **1997**, *16*, 67−80. (c) Nelson, P. S.; Muthini, S.; Kent, M. A.; Smith, T. H. *Nucleosides Nucleotides* **1997**, *16*, 1951−1959. (d) Nelson, P. S.; Muthini, S.; Vierra, M.; Acosta, L.; Smith, T. H. *BioTechniques* **1997**, *22*, 752−756. (e) Azhayev, A. V. *Tetrahedron* **1999**, *55*, 787−800.

(3) Universal supports derived from 3-amino-1,2-propanediol: (a) Lyttle, M. H.; Hudson, D.; Cook, R. M. *Nucleic Acids Res.* **1996**, *24*, 2793−2798. (b) Azhayev, A. V.; Antopolsky, M. L. *Tetrahedron* **2001**, *57*, 4977−4986.

(4) (a) Oivanen, M.; Kuusela, S.; Lönnberg, H. *Chem. Rev.* **1998**, *98*, 961−990. (b) Silnikov, V. N.; Vlassov, V. V. *Russ. Chem. Rev.* **2001**, *70*, 491−508.

(5) Connors, K. A. *Chemical Kinetics: The Study of Reaction Rates in Solution*; Wiley-VCH: Weinheim, Fed. Rep. Germany, 1990; pp 177−180.

(6) See the Supporting Information for a detailed discussion.

JA0284613