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Synthetic Method for Oligonucleotide Block by Using Alkyl-Chain-Soluble Support

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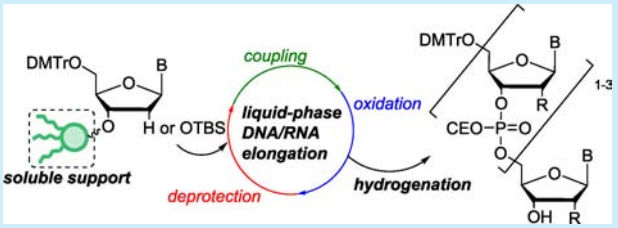
Supporting Information

ABSTRACT: A straightforward method for the synthesis of oligonucleotide blocks using a Cbz-type alkyl-chain-soluble support (Z-ACSS) attached to the 3′-OH group of 3′-terminal nucleosides was developed. The Z-ACSS allowed for the preparation of fully protected deoxyribo- and ribo-oligonucleotides without chromatographic purification and released dimer- to tetramer-size oligonucleotide blocks via hydrogenation using a Pd/C catalyst without significant loss or migration of protective groups such as 5′-end 4,4′-dimethoxtrityl, 2-cyanoethyl on internucleotide bonds, or 2′-TBS.



Chemical synthesis of oligonucleotides has provided numerous tools based on nucleic acids, such as nucleic acid drugs, fluorescent probes for gene detection, primers for PCR, and oligonucleotide fragments[1] for use in gene modification. In addition, oligonucleotide blocks have been utilized for combinatorial protein engineering based on oligonucleotide-directed mutagenesis[2] and in block condensation methods[3] for the preparation of long-mer DNAs and RNAs. In these applications, the oligonucleotide block is often used after conversion to phosphoramidite building block through phosphitylation of its 3′-OH group. Therefore, considerable effort has been devoted to developing efficient methods for the synthesis of oligonucleotide blocks in which the 3′-end-OH can be selectively exposed.[2c,d,3a,4]

Oligonucleotide blocks are commonly formed by selective deprotection of the 3′-end-OH in fully protected oligonucleotides prepared using a solution-phase approach.[4a,c−i] This synthetic strategy has led to the production of both deoxyribo- and ribo-oligonucleotide blocks; however, it requires repetitive chromatographic purification of the oligonucleotides as well as preparation of phosphoramidite monomers with a set of protecting groups that allow for selective deprotection of the 3′-end-OH. In addition, the protecting groups in such phosphoramidites are often resistant to basic conditions or fluoride anion treatment, resulting in the need to apply cumbersome methods for global deprotection of the final oligomer in which the oligonucleotide block was incorporated.[2c,3c,4b−i] These drawbacks have hampered development of methods for straightforward production and application of oligonucleotide blocks.

An ideal method for the preparation of oligonucleotide blocks might involve a support-synthetic approach[5] utilizing commercially available phosphoramidite reagents bearing a 4,4′-dimethoxytrityl (DMTr) group on the 5′-OH, a 2-cyanoethyl (2-CE) group on the phosphorus atom, and/or a TBS group on the 2′-OH.[4a] These oligonucleotide-protecting groups are labile to acidic, basic, oxidative, and fluoride treatment methods frequently used to cleave the synthetic support and linker moiety[6] on the 3′-end of oligonucleotides. As a result, the production of oligonucleotide blocks using synthetic supports is very difficult. Although UV irradiation is orthogonal to these conditions, it often causes troublesome production of byproducts, such as pyrimidine dimers.

In addressing this challenge, we hypothesized that deprotection of the Cbz moiety by hydrogenation[7] could be applied for selective cleavage of the 3′-end synthetic support. Hydrogenation of a Cbz moiety proceeds orthogonally to that of many other protective groups and is frequently employed in the deprotection of hydroxyl groups of nucleoside derivatives. Moreover, solid catalysts such as Pd/C and Pd black facilitate the purification of deprotected products. In addition, using a soluble molecule as a synthetic support[8] would allow for more effective interaction with solid catalysts, triggering hydrogenate cleavage as well as facile synthesis of oligonucleotides without the need for chromatographic purification. We previously reported alkyl-chain-soluble support (ACSS) and its application for oligonucleotide synthesis via the phosphoramidite method.[8a,b] ACSS disperses homogeneously in a reaction solvent, which allows efficient phosphoramidite coupling reaction on this support. Additionally, ACSS precipitates by addition of a polar solvent, which enables rapid purification of supported oligonucleotide only by filtration. Furthermore, as described in Figure 1, we assumed that use of ACSS as a protecting group for the 3′-end-OH in the protected oligonucleotide through the Cbz moiety would release the oligonucleotide block following hydrogenation,

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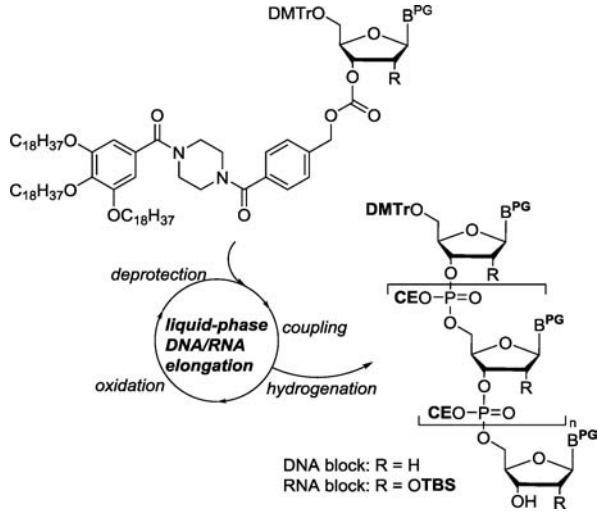


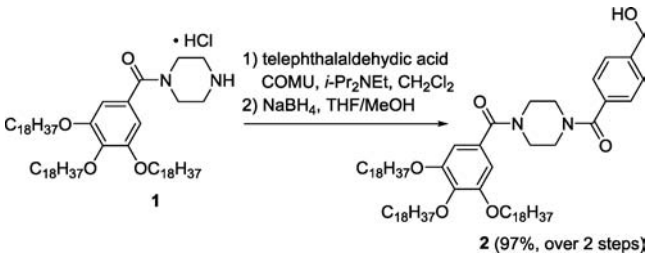
Figure 1. Schematic view of oligonucleotide block synthesis using Cbz-type alkyl-chain soluble support (Z-ACSS). PG: protecting group.

without loss of the protecting groups on the 5′-end-OH, the internucleotide bond, or the 2′-OH.

Herein, we report a method for the synthesis of oligonucleotide blocks using a Cbz-type alkyl-chain soluble support (Z-ACSS), which can be selectively cleaved via hydrogenation from oligonucleotides bearing 5′-DMTr, 2-CE, and/or 2′-TBS groups. We also demonstrate the utility of the method for the synthesis of dimer, trimer, and tetramer deoxyribo- and/or ribo-oligonucleotide blocks.

Initially, we prepared a Z-ACSS in which the protected oligonucleotide was attached, as described in Schemes 1 and 2.

Scheme 1. Synthesis of Soluble Support 2



Starting with **1**, the desired soluble support **2** was obtained at 97% yield in two steps via simple condensation using *N*-[1-(cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-(morpholino)uronium] hexafluorophosphate (COMU) and reduction using $NaBH_4$, without chromatographic purification. Soluble support **2** was condensed with activated deoxythymidine $\mathbf{3}^{dT}$ to form a carbonate linkage, affording the desired primary support $\mathbf{4}^{dT}$ at 97% yield. Oligonucleotide elongation was subsequently carried out using this primary support, as previously described,[8a,b] producing supported oligonucleotide **5a** at 98% yield. This result indicated that structural changes in the novel support do not affect the coupling efficiency of the phosphoramidite unit.

Next, we examined the selective cleavage of the Z-ACSS from a protected oligonucleotide with a DMTr group at the 5′-end position and a 2-CE group on the internucleotide bond and optimized the reaction conditions (Table 1). A solution of dimer deoxyribonucleotide **5a** was treated with a series of Pd catalysts under H_2 atmosphere at room temperature for 20 h,

Scheme 2. Synthesis of Z-ACSS-Supported Oligonucleotide 5a–i

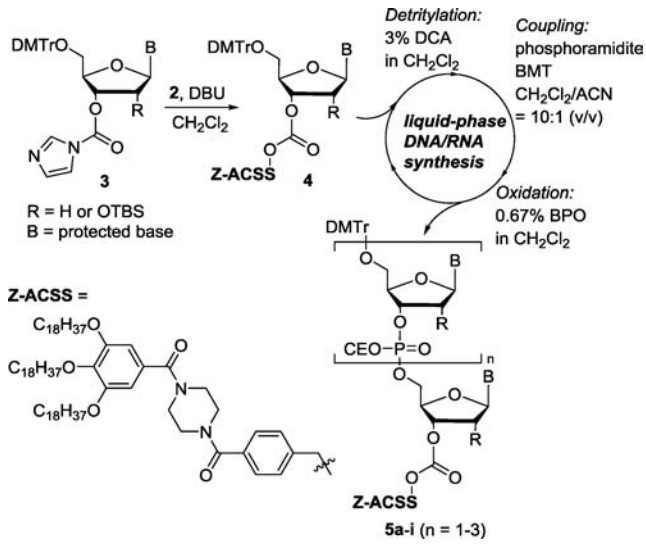
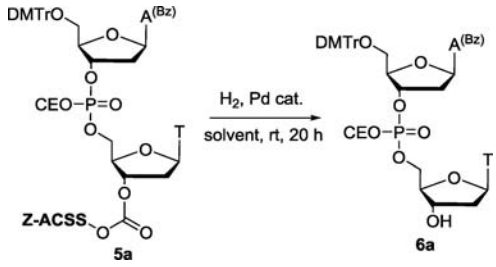


Table 1. Hydrogenate Cleavage of Supported DNA 5a



entry	catalyst	solvent	conv[a] (%)	yield of **6a**[b] (%)
1	Pd/C (5%)	THF	80	41
2	Pd/C (10%)	THF	96	51
3	Pd/C (20%)	THF	78	40
4	Pd black	THF	nr	
5	$Pd(OH)_2/C$ (20%)	THF	94	39
6	Pd/C (10%)	CH_2Cl_2	nr	
7	Pd/C (10%)	CH_2Cl_2:THF = 1:1 (v/v)	nr	
8	Pd/C (10%)	CH_2Cl_2:ACN = 10:1 (v/v)	58	18

[a]Determined by ^{1}H NMR, [b]Isolated yield.

and then the Pd catalyst was removed from the reaction mixture by centrifugation. The resulting solution was diluted with methanol to separate the remaining **5a**, cleaved support, and slightly remaining Pd catalyst by filtration from dimer deoxyribonucleotide block **6a**. The conversion rate of **5a** was determined by ^{1}H NMR spectroscopy, and the yield of isolated **6a** was also determined. Entries 1–4 showed that the Pd/C catalyst (5, 10, and 20%) was more effective for the cleavage of Z-ACSS in **5a** (80, 96, and 78% conversion rates, respectively) than Pd black (nr) after 20 h. This result indicated the surface area of the Pd catalyst is more critical than the amount of Pd present. In these reactions, no significant loss of DMTr, 2-CE, or the acyl protection groups on the nucleobases was observed, and no significant byproducts were produced due to selectivity of the hydrogenate cleavage reaction. The recovery yield of desired product **6a** was approximately 50%, probably because

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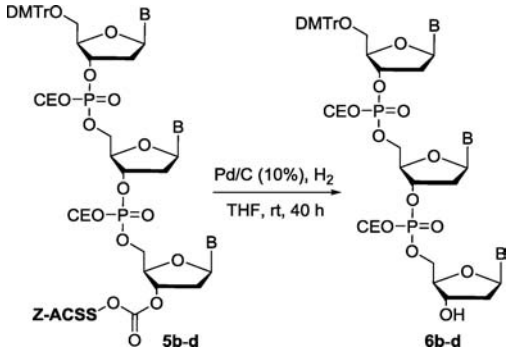
the oligonucleotide fragment was adsorbed on a charcoal of Pd/C and was lost during separation with the catalyst. Although the use of $Pd(OH)_2/C$ (20%) resulted in a high conversion rate (94%) in entry 5, the yield of **6a** was reduced because some of the DMTr groups were removed. Tetrahydrofuran (THF) gave good results during solvent screening. The use of CH_2Cl_2 and THF/CH_2Cl_2 = 1:1 (v/v) as a reaction solvent resulted in no reaction (entries 6 and 7). CH_2Cl_2/acetonitrile (ACN) = 10:1 (v/v) reaction solvent caused partial deprotection of the CEO group, having decreased the yield of **6a** (entry 8).

Additionally, our method produces no significant reduction of the C5−C6 double bond in pyrimidine bases (C and T), which previous studies have reported as a side reaction in the hydrogenation of pyrimidine nucleosides/nucleotides that is enhanced in the presence of protic solvents or under conditions of increasing pressure. This undesired reduction is suppressed in the present method because the hydrogenate cleavage of the Z-ACSS proceeds in aprotic solvents (THF in this study), without pressurization.

We also checked for leakage of Pd from a charcoal-supported catalyst using microwave plasma-atomic emission spectroscopy. Neither the Pd catalyst filtration supernatant nor purified dimer nucleotide block contained detectable amounts of Pd (see the Supporting Information).

We also evaluated the effects of oligonucleotide length and type of nucleoside at the 3′-end position on the efficiency of hydrogenation (Table 2). A series of supported trimer

Table 2. Synthesis of Trimer DNA Blocks



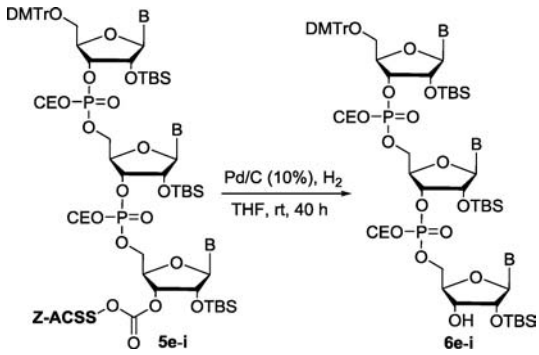
entry	sequence (3′→5′)	conv[a] (%)	yield of **6b−d**[b] (%)
1	$d(TA^{(Bz)}C^{(Bz)})$ (**b**)	75	44
2	$d(A^{(Bz)}TG^{(Dmf)})$ (**c**)	70	42
3	$d(G^{(Ib)}C^{(Bz)}A^{(Bz)})$ (**d**)	77	49

[a]Determined by ^{1}H NMR, [b]Isolated yield.

deoxyribo-oligonucleotides (**5b−d**) were prepared from primer supports $\mathbf{4}^{dT}$, $\mathbf{4}^{dA(Bz)}$, and $\mathbf{4}^{dG(Ib)}$, respectively, as described in Scheme 2. Hydrogenation of **5b−d** was carried out using Pd/C (10%) in THF, and the resulting mixtures were stirred for 40 h. We extended the reaction time in this examination because the conversion rate of **5b−d** and yields of **6b−d** were not satisfied at the 20 h time point. The conversion rates of **5b−d** and yields of **6b−d** were determined in the manner described in Table 1. Extension of the oligomer length resulted in a decrease in the conversion rate of supported substrate. This result suggests that extension of oligomer length hampers interaction between the 3′-end benzoylcarbonate moiety and the catalytic surface.

We also examined the hydrogenation of supported ribo-oligonucleotides **5e−i** under the same conditions, as described in Table 3. The conversion rates of trimer ribo-oligonucleotide **5e−h** were better than for deoxyribo-oligonucleotide, even

Table 3. Synthesis of Trimer and Tetramer RNA Blocks



entry	sequence (3′→5′)	conv[a] (%)	yield of **6e−i**[b] (%)
1	$r(UA^{(Bz)}C^{(Ac)})$ (**e**)	84	27
2	$r(C^{(Bz)}A^{(Bz)}G^{(Ib)})$ (**f**)	84	47
3	$r(A^{(Bz)}UG^{(Ib)})$ (**g**)	85	51
4	$r(G^{(Ib)}C^{(Ac)}A^{(Bz)})$ (**h**)	88	31
5	$r(UUA^{(Bz)}G^{(Ib)})$ (**i**)	83	45

[a]Determined by ^{1}H NMR, [b]Isolated yield.

though the TBS group was attached on the 2′-end-OH group, which lay around the 3′-end benzoylcarbonate bond. Only acetyl groups on the exocyclic amine of $rC^{(Ac)}$ were partially deprotected (about 40% of the recovered oligonucleotide blocks) during hydrogenation and/or purification (entries 1 and 4). The hydrogenation of **5i** gave tetramer oligonucleotide block **6i** at good yields, although the yields were lower than that of the trimer. This indicates that relatively longer oligonucleotides can also be easily obtained using our method. Further studies to determine the limitations and applicability of this method are in progress.

In conclusion, we developed a facile method for the synthesis of oligonucleotide blocks using a novel soluble support linked with the 3′-OH group of 3′-terminal nucleosides thorough a benzyloxycarbonate bond (Z-ACSS). The Z-ACSS allows preparation of supported oligonucleotides without repetitive purification. Furthermore, it released dimer to tetramer deoxyribo- and/or ribo-oligonucleotide blocks via hydrogenation using a Pd/C catalyst without significant deprotection or migration of protecting groups such as DMTr on the 5′-end-OH, 2-CE on internucleotide bonds, or TBS on 2′-OH groups.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00077.

Experimental procedures, compound characterization data, and ^{1}H, ^{13}C, and ^{31}P NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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