endoliposomal functional head groups to exoliposomal loci, whereas similar treatment of differentiated **1-F**, **2-F**, or **5-F** coliposomes brings about reequilibrations with $t_{1/2}$ = 2–5 min.

Even 1 h of heating at 60 °C occasions only 18% flip of **3-F** or **4-F**. This unprecendented[3,13] thermal stability for ammonium ion lipids, expressed as extraordinary resistance to transverse bilayer migration, reflects the inability of biphenyl-stiffened, bridging **3-F** or **4-F** to readily bend within the bilayer. Monopolar lipids, or the all-methylene bola **1-F** with no built-in barrier to bending, exhibit normal dynamics.

In bilayers, the biphenyl units of **3-F** and **4-F** inhibit bending in the middle of the bolas' main chains. However, *monolayers* of **3-NF**, like the natural bolaamphiphiles,[1a,d,e] do feature U-plan arrangements at the air/water interface.[14] The bending here must occur at either side of the biphenyl group.

Acknowledgment. We are grateful to Mr. J. Simon and Prof. H. Ringsdorf for monolayer experiments with **3-NF**. We thank the U.S. Army Research Office and the Busch Memorial Fund of Rutgers University for financial support.

Supplementary Material Available: Details of synthetic schemes for bolaamphiphiles **3-F** and **4-F** (2 pages). Ordering information is given on any current masthead page.

(13) Moss, R. A.; Fujita, T.; Okumura, Y. *Langmuir* **1991**, *7*, 440.
(14) Private communication from Mr. J. Simon and Prof. H. Ringsdorf, University of Mainz, Mainz, Germany.

Synthesis of a 4-Thio-2′-deoxyuridine-Containing Oligonucleotide. Development of the Thiocarbonyl Group as a Linker Element

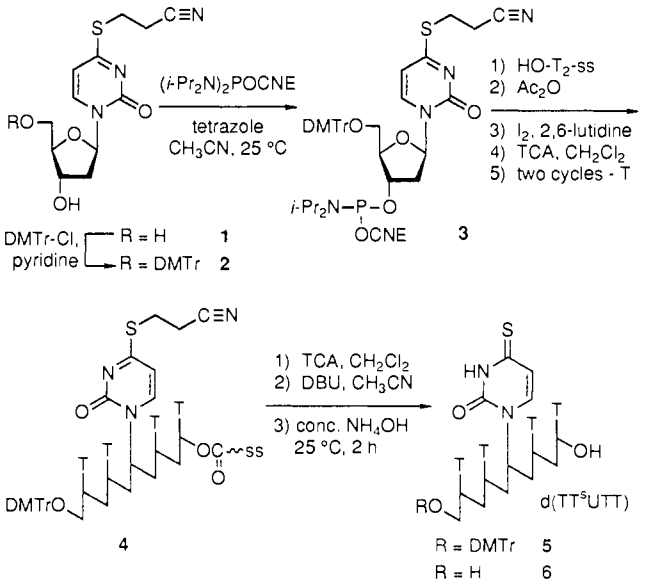
Robert S. Coleman*,[1] and James M. Siedlecki

Department of Chemistry and Biochemistry
University of South Carolina
Columbia, South Carolina 29208
Received July 9, 1992

The synthetic incorporation of non-natural functionality into oligonucleotides has provided a variety of templates upon which to tether reactive or reporter groups[2] such as chemically reactive species[3,4] or intercalating ring systems.[5] Various reports have described the synthesis and incorporation of "modified" nucleic acids into oligonucleotides;[2,6] the most flexible approaches have utilized a postsynthesis modification strategy. This tactic involves the incorporation of a functionalized non-natural nucleic acid into a growing oligonucleotide chain and is followed by chemical modification of the non-natural base. This makes possible the divergent incorporation of reactive functionality that would otherwise be incompatible with solid-phase synthesis conditions. Examples include reports by Webb and Matteucci[3a] and Verdine and co-workers[7] that describe the synthesis and postsynthetic modification of base-functionalized oligonucleotides. Herein, we report our preliminary results on the incorporation of 4-thio-2′-deoxyuridine residues into oligodeoxynucleotides,[8] and the development of the appendant thiocarbonyl group as a site-specific handle for the attachment of functionalized tethers.

The synthesis of thionucleic acid-containing oligonucleotides is hampered by the instability of the thiocarbonyl group to solid-phase synthesis conditions.[8a] We reported[9] an efficient synthesis of *S*-(2-cyanoethyl) 4-thio-2′-deoxyuridine (**1**) and detailed its stability to reagents used for oligonucleotide synthesis.[8b,10] An *S*-cyanoethyl ether allows for S-deprotection concomitant with removal of the cyanoethyl ester phosphate protecting groups.[10] Disulfide-based protecting groups were unsuitable, since the disulfide linkage labilized the carbon–sulfur thioimidate bond to hydrolysis. Other protecting groups[8a] and methods for incorporation of a thiocarbonyl group[11] have not proven effective.

Protection of **1** as the dimethoxytrityl (DMTr) ether (DMTrCl, pyridine, 25 °C, 87%) afforded **2** and was followed by phosphitylation[10] (tetrazole, $(i\text{-Pr}_2\text{N})_2\text{POCH}_2\text{CH}_2\text{CN}$, CH_3CN, 25 °C, 98%) to afford phosphoramidite **3**. Incorporation of **3** into a growing oligonucleotide chain was achieved using an Applied Biosystems 380B oligonucleotide synthesizer.[10] Thus, phosphitylation of the 5′-hydroxyl group of a solid support (ss) linked TT-dinucleotide with **3** was followed by standard end-capping (Ac_2O, 2,6-lutidine, THF), oxidation (I_2, H_2O/pyridine/THF), detritylation (2% CCl_3CO_2H (TCA) in CH_2Cl_2), and oligomer elongation with two additional thymidine residues to afford **4**. The *S*-cyanoethyl ether and *O*-cyanoethyl phosphate esters were removed by treatment with 1.0 M DBU in CH_3CN for 1 h.[12] Cleavage of the oligonucleotide from the solid support (concentrated NH_4OH, 25 °C, 2 h) afforded pentamers **5** and **6**. Yields for each coupling step were in excess of 94%. "Trityl-on" pentamer **6** could be purified by HPLC (1 × 25 cm C18 column, 0.1 M NH_4OAc, 1–50% CH_3CN/H_2O gradient, 4 mL/min). The purity of pentamers **5** and **6** was determined by ^1H NMR spectroscopy; no resonances were observed that were attributable to a uridine residue.



(1) Recipient of a Camille and Henry Dreyfus Foundation Distinguished New Faculty Award (1989-94) and an American Cancer Society Junior Faculty Research Award (1991-93).
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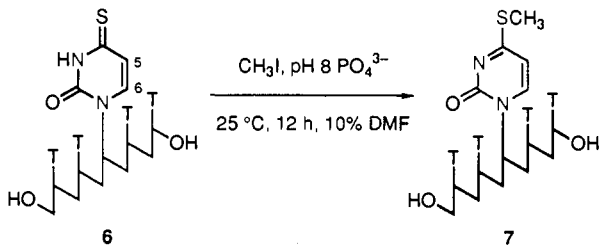
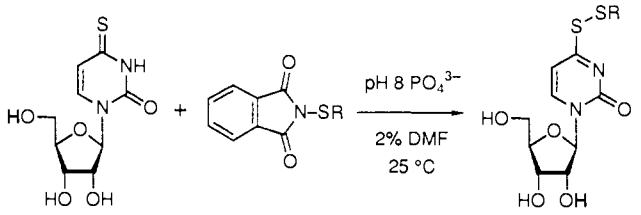
The thiocarbonyl group of **5** and **6** proved suitable for attachment of pendant groups. In studies utilizing 4-thiouridine, we observed that significant rates of S-alkylation[13] under aqueous conditions (50 mM pH 8 PO_4^{3-}, 10–30% DMF) required reactive electrophiles such as allylic or benzylic bromides. This methodology was applied by treatment of pentamer **6** with iodomethane (≈1 equiv) in 0.1 M pH 8 phosphate buffer (10% DMF) and afforded *S*-methyl thioimidate **7** in quantitative yield, as evidenced by the complete disappearance of the C5-H and C6-H signals of **6** in the ^1H NMR, which were replaced by two new signals corresponding to **7**.[14] Although S-alkylation of the thiocarbonyl group of **6** occurred quantitatively, it is not apparent whether this protocol for attachment of tethers will prove selective with oligonucleotides containing nucleophilic residues (e.g., G or A).



6 **7**

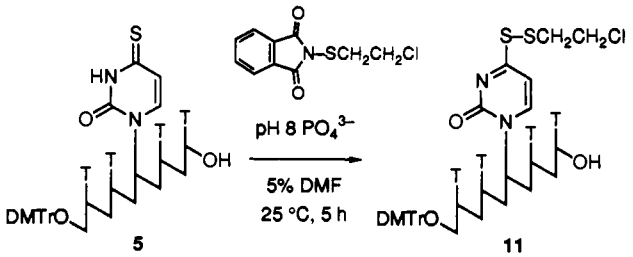
We developed a simple method for tether attachment that relied on selective mixed disulfide formation. Reaction of 4-thiouridine (**8**) with *N*-mercaptophthalimides **9a–d**[15,16] (1 equiv) in aqueous buffer containing 2% DMF (25 °C, 1 h) effected thiol-group transfer to afford mixed imino disulfides **10a–d** in ≥90% yields.



8

9a	R = CH_3	10a
9b	R = CH_2CH_2Cl	10b
9c	R = CH_2CH_2Br	10c
9d	R = $CH_2CH_2CH_2Cl$	10d

Similarly, treatment of pentanucleotide **5** with the thiol-transfer reagent *N*-((2-chloroethyl)thio)phthalimide (**9b**)[16] in phosphate buffer (pH 8) containing 5% DMF effected quantitative conversion to disulfide **11**. Effective conversion of **5** to **11** was evident in the ^1H NMR (500 MHz, D_2O) by the complete disappearance of the C5-H and C6-H signals of **5**, which were replaced by two new signals corresponding to **11**.[17] The transformation of **5** to **11** is anticipated to be selective for thioalkyl transfer to thiocarbonyl groups and, therefore, potentially more appropriate for tether attachment than S-alkylation.



5 **11**

We have demonstrated a convenient and effective protocol for the incorporation of 4-thio-2′-deoxyuridine into simple oligonucleotides. This procedure used an *S*-(2-cyanoethyl) ether[9] as a thiocarbonyl protecting group, which was shown to be completely stable to the reaction conditions used during solid-phase oligonucleotide synthesis. Quantitative S-deprotection was effected by treatment of the support-linked oligonucleotide with DBU in CH_3CN. Further studies illustrated that the thiocarbonyl group provides a convenient point of attachment of alkyl tethers by postsynthetic S-alkylation or mixed disulfide formation. This methodology will be of potentially general value in appending a variety of reactive or reporter groups to 4-thio-2′-deoxyuridine-containing oligonucleotides.

Acknowledgment. We thank the American Cancer Society (IRG-107P), the Camille and Henry Dreyfus Foundation (NF-89-18), and the Carolina Venture Fund for their generous support of this work. NMR spectra were obtained on instruments purchased with funds from the National Science Foundation (CHE-8411172 and CHE-8904942) and the National Institutes of Health (S10-RR02425).

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(17) Characteristic chemical shift values (500 MHz, D_2O): δ 6.46 (1 H, C5-H), 7.66 (1 H, partially obscured by thymidine, C6-H) for **5**; δ 7.05 (1 H, C5-H), 8.24 (1 H, C6-H) for **11**.

Hydrogen Trajectories in Alkene to Carbene Rearrangements. Unequal Deuterium Isotope Effects for the Axial and Equatorial Paths

Alex Nickon,*,† Martin C. Ilao,† Alfred G. Stern,† and Michael F. Summers‡

Department of Chemistry
The Johns Hopkins University
Baltimore, Maryland 21218
Department of Chemistry
University of Maryland—Baltimore County
Baltimore, Maryland 21228

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The rearrangement of a singlet carbene to an alkene is well-known, and its stereochemical aspects have been probed experimentally[1] and theoretically[2] for migration of H (**1** → **2**). The

† The Johns Hopkins University.
‡ University of Maryland—Baltimore County.
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