1-Acetoxy-2,3-diphenylpyrrolo[1,2-*a*]pyrimidine (30): mp 141-142 °C; ^{1}H NMR ($CDCl_3$) δ 8.24 (dd, J = 7.3, 1.3 Hz, 1 H), 8.08 (dd, J = 3.6, 1.3 Hz, 1 H), 7.45-7.20 (m, 10 H), 6.47 (dd, J = 7.3, 3.6 Hz, 1 H), 2.33 (s, 3 H); IR (KBr) 1765, 1602, 1510, 1440, 1368, 1208, 1074, 769 cm^{-1}; FDMS m/e 328. Anal. Calcd for $C_{21}H_{16}N_2O_2$: C, 76.81; H, 4.91; N, 8.53. Found: C, 76.68; H, 5.09; N, 8.45.

1,10-Diacetoxy-2,3,8,9-tetraphenylpyrrolo[1,2-*a*]pyrrolo-[1,2-*c*]pyrazine (4). A solution of pyrazine (0.40 g, 5.0 mmol) and **1** (2.06 g, 10.0 mmol) in 20 mL of *p*-dioxane was refluxed under argon for 20 min. When the solution was cooled, a crystalline precipitate separated and was filtered to afford the free diol of **4**. This material was directly treated with acetic anhydride (2.04 g, 20 mmol) in 10 mL of pyridine, and the mixture was heated at 100 °C for 10 min. The mixture was poured into water, and the resulting precipitate was filtered and recrystallized from methanol to provide compound **4** (2.16 g, 75%) as a pale tan solid: mp 271 °C; ^{1}H NMR ($CDCl_3$) δ 7.40-7.17 (m, 20 H), 6.98 (s, 2 H), 2.25 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 169.2, 131.5, 130.0, 129.2, 128.9, 128.0, 127.4, 127.3, 127.2, 125.8, 123.0, 118.4, 112.0, 108.3, 20.2; IR (KBr) 1770, 1360, 1175 cm^{-1}; FDMS m/e 576. Anal. Calcd for $C_{38}H_{28}N_2O_4$: C, 79.15; H, 4.89; N, 4.86. Found: C, 78.66; H, 5.01; N, 4.78.

3-Acetoxy-1,2-diphenylpyrrolo[1,2-*a*]quinoline (26). Diphenylcyclopropenone **1** (1.02 g, 5.0 mmol) was added to 10 mL of quinoline that had been thoroughly purged with argon for 5 min. This mixture was heated at 90 °C until an infrared spectra indicated the disappearance of the 1850 cm^{-1} cyclopropenone band. Acetic anhydride (1.02 g, 10.0 mmol) and pyridine (0.8 g, 10.0 mmol) were then added, and the mixture was warmed at 90 °C for 10 min. The reaction mixture was then dripped into a large volume of dilute HCl and the precipitated produced filtered off and recrystallized from methanol/water to provide 1.06 g (56%) of **26** as a yellow solid: mp 150-151 °C; ^{1}H NMR ($CDCl_3$) δ 7.58 (d, J = 7.6 Hz, 1 H), 7.50-7.00 (m, 15 H), 2.28 (s, 3 H); IR (KBr) 1763, 1604, 1360, 1199 cm^{-1}; FDMS m/e 377. Anal. Calcd for $C_{26}H_{19}NO_2$: C, 82.74; H, 5.07; N, 3.71. Found: C, 82.51; H, 5.26; N, 3.59.

Preparation of Azaindolizinol Ethers. The preparation of the azaindolizinol ethers **9**, **12**, and **15** were performed similarly to the literature procedure.[3] The preparation of **9** is illustrative.

2,3-Diphenyl-1-ethoxy-6-methylpyrrolo[1,2-*a*]pyrazine (9). A suspension of **7** (0.3 g, 1.0 mmol) in 10 mL dry methylene chloride was purged with argon. The mixture was treated with 2 equiv of triethyloxonium tetrafluoroborate (1 M in methylene chloride, 2.0 mL, 2.0 mmol), whereupon the mixture became homogeneous. This was stirred at room temperature for 30 min and washed with dilute Na_2CO_3 and water, and the organic layer was dried over $MgSO_4$. Concentration in vacuo provided 0.33 g (100%) of crude product, which was recrystallized from methanol/water to provide pure **9**: mp 158 °C (lit.[3] mp 157 °C); ^{1}H NMR ($CDCl_3$) δ 8.80 (s, 1 H), 7.54 (s, 1 H), 7.50-7.10 (m, 10 H), 3.95 (q, J = 7.0 Hz, 2 H), 2.32 (s, 3 H), 1.29 (t, J = 7.0 Hz, 3 H).

6,8-Dimethyl-2,3-diphenyl-1-ethoxypyrrolo[1,2-*a*]pyrazine (12): 77% yield; mp 124-126 °C (lit.[3] mp 62 °C);[9] ^{1}H NMR ($CDCl_3$) δ 7.45-7.10 (m, 11 H), 3.73 (q, J = 7.0 Hz, 2 H), 2.81 (s, 3 H), 2.27 (s, 3 H), 1.22 (t, J = 7.0 Hz, 3 H).

1,2-Diphenyl-3-ethoxy-5-methylpyrrolo[1,2-*a*]quinoxaline (15): 83% yield; mp 155-156 °C (lit.[3] mp 152 °C); ^{1}H NMR ($CDCl_3$) δ 7.78 (d, J = 7.9 Hz, 1 H), 7.45-7.10 (m, 11 H), 7.03-6.90 (m, 2 H), 3.77 (q, J = 7.0 Hz, 2 H), 2.90 (s, 3 H), 1.25 (t, J = 7.0 Hz, 3 H).

Supplementary Material Available: Difference NOE spectra for relevant compounds, HETCOR 2D NMR spectra for **6**, and ^{1}H NMR spectra of the Eu(fod)$_3$ shift reagent experiment of **26** (23 pages). Ordering information is given on any current masthead page.

(9) Compound **12** was observed to partially melt at 60-65 °C and then resolidify and remelt sharply at 124-126 °C.

Palladium-Mediated Synthesis of C-5 Pyrimidine Nucleoside Thioethers from Disulfides and Mercurinucleosides

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Thioether-linked side chains can be created at C-5 of pyrimidine nucleosides via a palladium-mediated reaction of mercurated nucleosides with organic disulfides. 5-(Chloromercuri)-2′-deoxyuridine reacts with butyl disulfide, phenyl disulfide, dimethyl 3,3′-dithiodipropionate, and *N,N′*-bis(trifluoroacetyl)cystamine to yield respectively 5-(1-thiapentyl)-2′-deoxyuridine, 5-(phenylthio)-2′-deoxyuridine, 5-[3-(methoxycarbonyl)-1-thiapropyl]-2′-deoxyuridine, and 5-[3-(trifluoroacetamido)-1-thiapropyl]-2′-deoxyuridine in yields ranging from 46 to 73%. Other mercurated nucleosides, including 5-(chloromercuri)-2′-deoxycytidine, 5-(chloromercuri)cytidine, and 5-(chloromercuri)tubercidin react with *N,N′*-bis(trifluoroacetyl)cystamine and lithium-palladium chloride in methanol to yield the corresponding coupled products, but the yields are much lower (5-10%). The nucleoside coupling reaction is complicated by competing side reactions between disulfides and Pd^{2+}, which remain to be elucidated.

Introduction

Nucleic acid components modified at C-5 of pyrimidine occur frequently in nucleic acids. Hypermodification at C-5 occurs in the DNA of bacteriophages such as SP-15 (*Bacillus subtilis*),[1] T-even phages (*Escherichia coli*),[2-4] and φω-14 (*Pseudomonas acidovorans*).[5] C-5 methylation of specific deoxycytidines in DNA playes a role in recognition and function of DNA binding molecules. At least nine different C-5-modified uridines occur in tRNA.[6] 2′-Deoxyuridine analogues having substituents at C-5 such as ethyl, iodo, propenyl, and bromoethenyl are selective anti-herpes agents.[7] C-5-substituted 2′-deoxyuridine monophosphates, having relatively small but highly elec-

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0022-3263/91/1956-5598$02.50/0

tronegative substituents, are potent inhibitors of dTMP synthetase.[8]

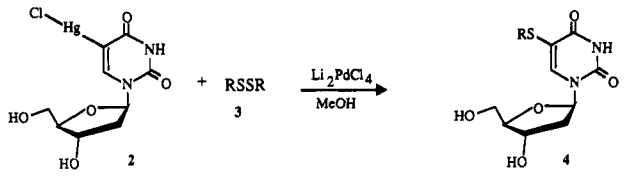
In recent years, there has been significant interest in the potential of C-5 as a tether site for linking reporter groups to nucleic acids. As a result, methodology for constructing suitable linker arms and generating bonds to C-5 have been extensively investigated. This is exemplified by the studies on the synthesis of alkynyl derivatives of dU.[9,10] The most widely used method for generating C-5 linkers has been the palladium-mediated reaction of 5-iodo-2′-deoxyuridine and 5-(chloromercuri)-2′-deoxyuridine originally reported by this laboratory.[11] For example, this methodology has been utilized by Leary et al. for the preparation of biotin-labeled DNA probes,[12] Dreyer and Dervan to link an iron–EDTA to an oligonucleotide,[13] Jablonski et al. to prepare oligodeoxyribonucleotide phosphatase conjugates,[14] Telser et al. to construct oligomers with tris(2,2′-bipyridine)ruthenium(II),[15] 9,10-anthraquinone, pyrenebutyrate, and pyrenesulfonate attached,[16] Bashkin et al. to prepare nucleoside–peptide conjugates,[17] and Iverson and Dervan to synthesize oligodeoxyribonucleotide methyl thioether probes.[18]

Although many other sites can be utilized in the preparation of conjugates of modified oligonucleotides,[19] C-5 is unique in that (1) the substituents are positioned in the major groove on double helix formation, (2) there is little interference with base pairing, and (3) the modification can be placed at virtually any position occupied normally by thymidine. For the purpose of constructing oligonucleotide conjugates, it is by no means necessary for the tether to be linked via a carbon–carbon bond at C-5. Other linkages that are stable to the usual chemical steps needed to construct modified oligonucleotides by way of phosphoramidite, H-phosphonate, or enzymic methodology may serve as well. Of course, the linkage must be sterically acceptable and should not alter the base pairing characteristics of the T (or U) it replaces.

For tethering most probes, a group such as $C^{5'}$—C≡C- or C^{5}—C=C appears to be nearly ideal, since these project directly out of the major groove with minimal steric interference. On the other hand, where the probe is designed to interact with a complementary sequence, a different type of linkage and scaffolding may be necessary. Finally, ease of synthesis is frequently a criteria in probe design. Other types of linkages may be preferable on this basis.

Although a number of synthetic routes to pyrimidine bases or nucleosides substituted at C-5 by thioether groups have appeared in the literature, none of these appear as versatile as the palladium-mediated reaction that forms the basis of this study. Cysteine reacts photochemically with uracil in low yield to give 5-*S*-cysteinyl-6-hydrouracil,[20] while cystamine reacts photochemically with 5-iodouracil to yield 5-*S*-cystaminyluracil.[21] Bardos and co-workers have described the synthesis of a number of *S*-alkyl derivatives of 2′-deoxyuridine by alkylation of 5-mercapto-2′-deoxyuridine with reactive bromides such as allyl bromide and 2-bromoacetamide.[22] More recently, Hayakawa et al. have described the preparation of 5-(phenylthio)uridine by the reaction of phenyl disulfide with the 5-lithiated derivative of 2′,3′,5′-tris-*O*-(*tert*-butyldimethylsilyl)uridine.[23] This reaction is limited by the requirement for protection and deprotection, the need to avoid groups that are reactive toward organolithium reagents, and the problem of obtaining both the 5- and 6-substituted isomers in the reaction. Finally, Chattopadhyaya and co-workers have reported the synthesis of a series of C-5 arenesulfenyl uridine derivatives by the reaction of arenesulfenyl chloride with 2′,3′,5′-tri-*O*-acetyluridine.[24]

Scheme I



		R		%Yield
3a	$(CH_3(CH_2)_3S)_2$	4a	$-(CH_2)_3CH_3$	73
3b	$(C_6H_5S)_2$	4b	$-C_6H_5$	52
3c	$(CH_3OC(O)CH_2CH_2S)_2$	4c	$-CH_2CH_2CO_2CH_3$	46
3d	$(CF_3C(O)NHCH_2CH_2S)_2$	4d	$-CH_2CH_2NHCCF_3$ (O)	51

Results and Discussion

5-(Chloromercuri)-2′-deoxyuridine reacts with disulfides and Li_2PdCl_4 in methanol at room temperature to yield two products, 2′-deoxyuridine and the C-5 (alkylthio)- or (arylthio)-2′-deoxyuridine (Scheme I). Although the number of successful reactions is rather limited, the examples illustrates that both aliphatic and aromatic disulfides couple and that protected polar functional groups (carboxylic acid as its methyl ester and aliphatic amine as its trifluoroacetyl derivative) are tolerated.

Of the disulfides investigated in this study, **3d** was of the greatest interest, since it would ultimately provide a free amino group that could be derivatized further should nucleoside **4d** be incorporated into an oligonucleotide. For this reason, the scope of the reaction of **3d** with other mercurated nucleosides was investigated. Three additional disulfides for which coupled products were not isolated include 2,2′-dithiodiethanol [$(HOCH_2CH_2S)_2$], cystamine dihydrochloride [$(Cl^-NH_3{}^+CH_2CH_2S)_2$], and 3,3′-dithiodipropionic acid [$(HO_2CCH_2CH_2S)_2$]. The failure of cystamine to react was not unexpected, since it was anticipated that this compound would form chelates with Pd^{2+}. 3,3′-Dithiodipropionic acid did yield coupled product on

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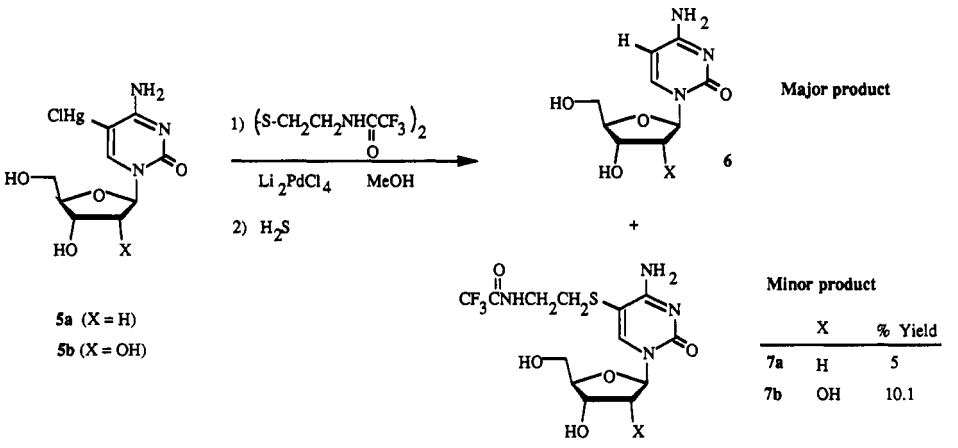
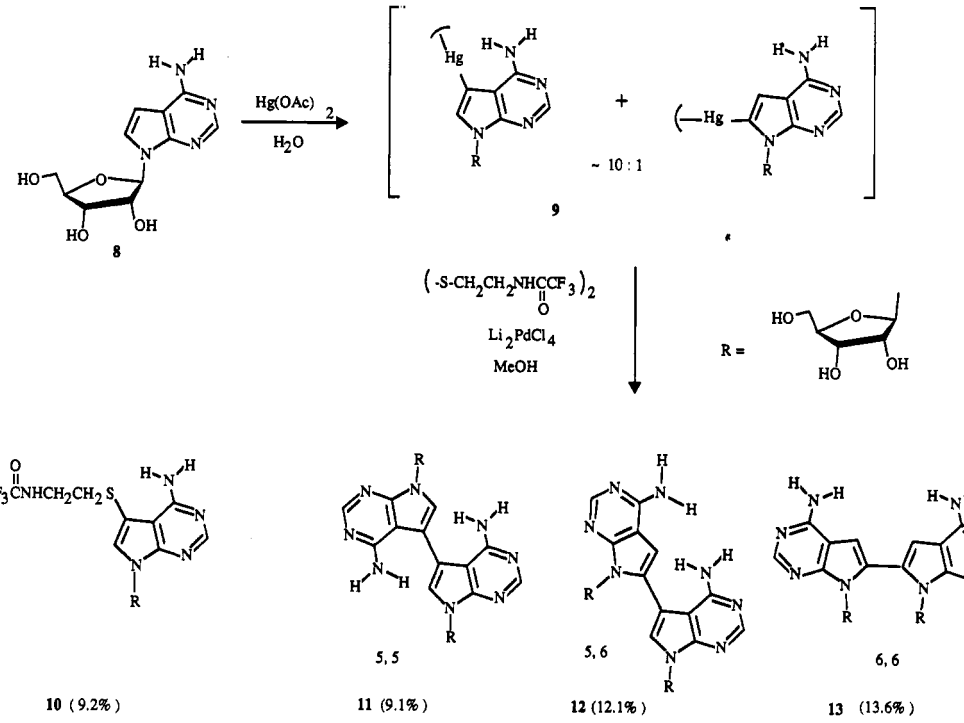
Scheme II



Scheme III



the basis of spectroscopic analysis (NMR), but the product proved difficult to purify and was not pursued further. Most unexpected was the result with 2,2′-dithiodiethanol, where not even a trace of coupled product was detected.

To obtain an optimum yield of the coupled product, at least 2 equiv of Li_2PdCl_4 per equiv of mercurideoxyuridine was required. Typically, the reaction was also run with $2\ ^1/_2$ equiv of the disulfide. Less palladium or disulfide relative to mercurideoxyuridine gave lower yields, but larger excesses of the reagents did not appear to improve yields further.

The reaction of mercurated cytosine nucleosides **5a** and **5b** with *N,N′*-bis(trifluoroacetyl)cystamine was investigated (Scheme II). The yields of thioether-substituted nucleoside were very low (5–10%), and the principal product in each instance appeared to be the parent nucleoside, cytidine, and 2′-deoxycytidine. We had previously observed that the palladium-mediated reaction of 5-(chloromercuri)cytidine and 5-(chloromercuri)-2′-deoxycytidine with alkenes was slower than the corresponding reaction with 5-(chloromercuri)-2′-deoxyuridine. The slow reaction between the mercurated cytidines and palladium(II) may not compete effectively with the relatively fast palladium-mediated decomposition of disulfides.

The palladium-mediated reaction of (chloromercuri)-tubercidin with alkenes yielded C-5-substituted tubercidin derivatives,[25] but the yields were typically lower than those for the equivalent palladium-mediated reactions of 5-(chloromercuri)-2′-deoxyuridine. This also seemed to be the case for the reaction of (chloromercuri)tubercidin with *N,N′*-bis(trifluoroacetyl)cystamine (Scheme III). The major products were the dimers **11**, **12**, and **13**. Reaction of (chloromercuri)tubercidin with methanolic lithium–palladium chloride in the absence of disulfide gives these dimers as the only isolated products. The palladium-mediated dimerization of 5-mercuripyrimidines has also previously been demonstrated to yield both C-5- and C-6-coupled products.[26]

One can speculate from the above studies that the palladium-mediated disulfide coupling reaction is not likely to be useful for C-5 modification of cytidine or tubercidin

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unless the rate of the reaction can be increased in order to compete with the disulfide decomposition. A major limitation may be the lack of solubility of the mercurinucleosides, which would limit the rate of the Pd–Hg exchange reaction. Iodide ion is known to form water-soluble complexes with organomercurials. When sodium iodide and 5-(chloromercuri)-2′-deoxyuridine were combined, a water (or methanol with a few percent water added) soluble product was obtained. However, reaction of this soluble complex with *N,N′*-bis(trifluoroacetyl)cystamine and Li_2PdCl_4 in aqueous methanol did not improve the yield of the reaction.

Reaction between phenylmercuric chloride and *n*-butyl disulfide was also attempted. Following the usual reaction conditions and workup (H_2S), no new products were observed. Similar reaction of 1-(chloromercurio)-9-fluorenone also gave only the parent compound, 9-fluorenone.

Mechanistic studies up to this point have been rather limited, and it would be premature to speculate how the reaction occurs. However, some pertinent observations show that this is a unique reaction unlike other palladium-mediated reactions of sulfur-containing compounds reported in the literature. It was established at the outset that Pd(II) is an absolute requirement. No reaction occurs in its absence. Consequently, there is no direct exchange reaction with the disulfide similar to that observed between halogens and arylmercury compounds (leading to aryl halides). The disulfide was also an absolute requirement; reactions attempted with thiol in place of disulfide gave immediate precipitation of a metal–thiolate complex, and no coupled product was formed. Unlike the reaction between 5-ClHg-2′-dU (**2**), Pd(II), and olefins, which results in the precipitation of Pd(0) during the course, no precipitant is observed in the disulfide reaction until workup with hydrogen sulfide.

Although the palladium-mediated disulfide reaction may share some similarities with the palladium-catalyzed reaction described by Migita and co-workers,[27] the differences in oxidation states of the reactants suggest that there must be some major differences in mechanism. In fact, a reaction between benzenethiol and 5-iodo-2′-deoxyuridine mediated by $Pd(PPh_3)_4$ attempted under conditions similar to those used by Migita and co-workers failed, yielding mainly 2′-deoxyuridine and only a trace of product **4b**.

To test whether the palladium-mediated disulfide reaction might proceed via RSCl generated in situ, we attempted a reaction between dUHgCl and PhSCl in MeOH. 5-PhSdU was formed in barely detectable amounts; hence, we do not believe that sulfenyl chlorides are intermediates.

In summary, it is apparent that the palladium-mediated disulfide coupling reaction described in this paper is a new type of reaction for generating thioether linkages. However, there are limitations to its potential utility. Not enough structural variations in the arylmercury component have been investigated to know the full scope of the reaction. Some very common substances, such as phenylmercuric chloride, did not yield product. Even within the area of nucleoside chemistry, the only major nucleoside component that coupled effectively was 2′-deoxyuridine. The yield of coupled product from 2′-deoxycytidine, cytidine, and tubercidin was too low to consider the method of preparative value. Nonetheless, the reaction is a very effective way to tether groups to C-5 of 2′-deoxyuridine, and this class of compound is proving to play a major role in the engineering and construction of modified oligonucleotides for biochemical tools. Further development of the reaction, which will be detailed in forthcoming papers, has shown that more complex disulfides containing aromatic heterocyclic molecules and additional amide linkages couple effectively.

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Experimental Section

^1H and ^{13}C NMR were recorded in d_4-methanol or d_6-dimethyl sulfoxide with tetramethylsilane as the internal standard. Column chromatography was done on E. M. Science kieselgel 60 (70–230 mesh). Analytic thin layer chromatography was carried out on E. Merck glass or plastic baked precoated silica gel F-254 (0.25 mm) plates. Solvent systems for TLC or column chromatography were (a) CH_3OH–$CHCl_3$ (10:90 v/v); (b) CH_3OH–$CHCl_3$ (15:85 v/v); (c) CH_3OH–$CHCl_3$ (1:3 v/v); (d) CH_3OH–$CHCl_3$ (1:1 v/v); (e) MeCN–*n*–BuOH–0.1 M NH_4OAc–concd NH_4OH (1:6:2:1 v/v). For reference, 2′-deoxyuridine has an R_f of 0.19 in solvent system (a). All solvents and reagents were reagent grade.

Palladium chloride was obtained from Johnson Matthey. The 0.1 M solution of Li_2PdCl_4 in methanol was made by stirring a suspension of 1 equiv of $PdCl_2$ and 2 equiv of dry LiCl in the appropriate amount of methanol overnight. 2′-Deoxyuridine was purchased from United States Biochemical Corporation. Cystamine dihydrochloride and trifluoroacetic anhydride were purchased from Aldrich Chemical Company. Mercurinucleosides were synthesized by literature procedures.[28,29] *N,N′*-Bis(trifluoroacetyl)cystamine was synthesized from cystamine dihydrochloride and trifluoroacetic anhydride.

General Procedure for the Synthesis of (5-*RS*)-2′-Deoxyuridine. Finely ground 5′-(chloromercuri)-2′-deoxyuridine (0.926 g, 2.0 mmol), the disulfide (5 mmol), and a 0.1 M solution of Li_2PdCl_4 in methanol (40 mL) were combined in a round-bottom flask and stirred (magnetic stir bar) at ambient temperature for 14–16 h. The solution turns orange to yellow shortly after the reagents are combined and usually yielded a clear yellow-orange solution within a few hours. The reaction mixture was worked up by rapidly bubbling H_2S through the solution for 30 s. The solution obtained on gravity filtration was evaporated in vacuo to give an oil that was purified on a silica gel column, eluting with a linear chloroform/methanol gradient that varied from 10 to 18% methanol. Fractions containing the high R_f material were combined and evaporated to obtain the product.

5-(1-Thiapentyl)-2′-deoxyuridine (4a). Yield 73%. mp: 120–121 °C. Mass spectrum: mol. ion + Li = 323 (calcd 323). ^1H NMR (d_6-DMSO, δ): 8.29 (s, 1 H, H6), 6.32 (dd, 1 H, H1′, J = 6.5 Hz), 4.43 (m, 1 H, H3′), 3.99 (m, 1 H, H4′), 3.81 (m, 2 H, H5′), 2.74 (t, 2 H, H2″, J = 7.5), 2.28 (m, 2 H, H2′), 1.53 (m, 4 H, H3″, H4″), 0.95 (t, 3 H, H5″, J = 6.3 Hz). ^{13}C NMR (d_6-DMSO, δ): 164.4 (C4), 151.9 (C2), 144.1 (C6), 109.2 (C5), 89.0 (C1′), 86.8 (C4′), 72.2 (C3′), 62.6 (C5′), 41.6 (C2′), 33.9 (C3″), 32.1 (C2″), 22.6 (C4″), 14.0 (C5″). Anal. Calcd for $C_{13}H_{19}N_2O_5S$: C, 49.37; H, 6.33. Found: C, 49.52; H, 6.83.

5-(Phenylthio)-2′-deoxyuridine (4b). Yield: 52%. mp: 180–181 °C. Mass spectrum: 343 (calcd 343). ^1H NMR (d_6-DMSO, δ): 8.48 (s, 1 H, H6), 7.28 (m, 5 H, H2″), 6.28 (t, 1 H, H1′, J = 6.4 Hz), 4.42 (m, 1 H, H3′), 3.98 (m, 1 H, H4′), 3.75 (m, 2 H, H5′), 2.30 (m, 2 H, H2′). ^{13}C NMR (d_6-DMSO, δ): 164.0 (C4), 151.9 (C2), 147.8 (C6), 137.1, 130.0, 129.0, 127.3 (phenyl), 107.2 (C5), 89.0 (C1′), 87.1 (C4′), 72.0 (C3′), 62.5 (C5′), 41.7 (C2′). Anal. Calcd for $C_{14}H_{16}N_2O_5S$: C, 53.57; H, 4.76; N, 8.33. Found: C, 52.94; H, 4.86; N, 8.07.

5-[3-(Methoxycarbonyl)-1-thiapropyl]-2′-deoxyuridine (4c). Yield: 46%. mp: 148–149 °C. Mass spectrum: 353 (calc 353). ^1H NMR (d_6-DMSO, δ): 8.16 (s, 1 H, H6), 6.13 (dd, 1 H, H1′, J = 6.3 Hz), 4.22 (m, 1 H, H3′), 3.98 (m, 1 H, H4′), 3.90 (m, 2 H, H5′), 3.83 (s, 3 H, H6″), 2.98 (m, 2 H, H3″), 2.61 (t, 2 H, H2″, J = 7.0 Hz), 2.32 (m, 2 H, H2′). ^{13}C NMR (d_6-DMSO, δ): 174.0 (C4″), 164.4 (C4), 151.9 (C2), 145.9 (C6), 107.6 (C5), 89.1 (C1′), 86.9 (C4′), 72.1 (C3′), 62.7 (C5′), 52.3 (C6″), 41.6 (C2′), 34.7 (C2″), 29.4 (C3″). Anal. Calcd for $C_{13}H_{18}N_2O_7S$: C, 45.09; H, 5.20; N, 8.09. Found: C, 45.06; H, 5.46; N, 7.56.

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5-[3-(Trifluoroacetamido)-1-thiapropyl]-2′-deoxyuridine (4d). Yield: 51%. mp: 160.5–161 °C. Thin layer chromatography: R_f = 0.30 (a) or R_f = 0.71 (c). FAB MS (m/e): (M + 1) calcd 400.0770, found 400.0790. ^{1}H NMR (CD_3OD, δ): 8.32 (s, 1 H, H6), 6.27 (t, 1 H, J = 6.6 Hz, H1′), 4.43 (m, 1 H, H3′), 3.95 (m, 1 H, H4′), 3.79 (m, 2 H, H5′), 3.47 (t, 2 H, J = 6.0 Hz, H3″), 2.87 (m, 2 H, H2′), 2.32 (t, 2 H, J = 6.0 Hz, H2″). ^{13}C NMR (CD_3OD, δ): 164.5 (C4), 159.0 (C5″, q, J = 41.9 Hz), 151.9 (C2), 146.4 (C6), 117.4 (C6″, q, J = 286.6 Hz), 106.8 (C5), 89.0 (C1′),, 86.8 (C4′), 72.0 (C3′), 62.7 (C5′), 41.4 (C2′), 39.7 (C3″), 33.5 (C2″). IR (KBr): 3550–2900 (br O–H), 3423, 3343 (N–H), 1728, 1692, 1651, (C=O), 1660, 1553, 1461 (C=C), 1179, 1271 cm^{-1}. Anal. Calcd for $C_{13}H_{16}F_3O_6N_3S$: C, 39.08; H, 4.01; N, 10.52; S, 8.03. Found: C, 39.35; H, 3.63; N, 10.38; S, 8.22.

Reaction of 5-Mercuritubercidin with *N,N′*-Bis(trifluoroacetyl)cystamine. 5-Mercuritubercidin (0.5 mmol, 258.5 mg) and *N,N′*-bis(trifluoroacetyl)cystamine (1.25 mmol, 430 mg) were added to a 100-mL round-bottom flask containing a 0.1 M solution of Li_2PdCl_4 in methanol (1 mmol, 10 mL). An additional 10 mL of a 0.1 M solution of Li_2PdCl_4 (1 mmol) was added 3 h later. Then the mixture was stirred for 3 days and worked up with H_2S. The brown-black precipitate that formed was filtered and the solvent removed by rotary evaporation. The residue was redissolved in a small amount of methanol and coevaporated with 3 g of silica gel. Column chromatography (silica gel, 50 g, mobile phase c or d) was used to separate the coupled product **10** (20 mg), tubercidin **8** (50 mg), the mixture of 5,5, 5,6, and 6,6-, dimers **11, 12, 13**, and unreacted disulfide. The mixture of **11, 12, 13** was isolated by preparative TLC (solvent e). TLC: **10** R_f = 0.54; **8** R_f = 0.28 (solvent c); **11** R_f = 0.36; **12** R_f = 0.40; **13** R_f = 0.44 (solvent e).

10. Yield: 9.2%. ^{1}H NMR (CD_3OD, δ): 8.09 (s, 1 H, H2), 7.58 (s, 1 H, H6), 6.02 (d, 1 H, J = 6.2 Hz, H1′), 4.61 (m, 1 H, H4′), 4.29 (m, 1 H, H2′), 4.11 (m, 1 H, H2′), 3.80 (m, 2 H, H5′), 3.45 (t, 2 H, J = 6.7 Hz, H3″ disulfide), 2.85 (t, 2 H, J = 6.7 Hz, H2″ disulfide). ^{13}C NMR (CD_3OD, δ): 159.3 (C4), 159.0 (q, J = 41.9, C5″), 152.8 (C2), 151.1 (C7a), 131.4 (C6), 117.4 (q, J = 286.6 Hz, C6″), 105.5 (C4a), 103.7 (C5), 91.0 (C1′), 87.3 (C4′), 75.5 (C2′), 72.3 (C3′), 63.4 (C5′), 39.4 (C3″), 36.9 (C2″). FAB MS (m/e): $C_{15}H_{18}F_3N_5O_5S$ (M + 1) calcd 438.1059, found 438.1050.

11. Yield: 9.1%. ^{1}H NMR (CD_3OD, δ): 8.15 (s, 2 H, H2), 7.46 (s, 2 H, H6), 6.12 (d, 2 H, J = 6.5 Hz, H1′), 4.70 (m, 2 H, H4′), 4.32 (m, 2 H, H2′), 4.15 (m, 2 H, H3′), 3.82 (m, 4 H, H5′). FAB MS (m/e): $C_{22}H_{26}N_8O_8$ (M + 1) calcd 531.1952, found 531.1962.

12. Yield: 12.1%. ^{1}H NMR (CD_3OD, δ): 8.17 (s, 1 H, H2), 8.10 (s, 1 H, H2′), 7.64 (s, 1 H, H6′), 6.73 (s, 1 H, H5), 6.19 (d, 1 H, J = 6.4 Hz, H1′), 5.94 (d, 1 H, J = 6.4 Hz, H1″), 4.70 (m, 2 H, H4″, H4′), 4.33–4.25 (m, 2 H, H2″, H2′), 4.12 (m, 2 H, H3″, H3′), 3.84 (m, 4 H, H5″, H5′). FAB MS (m/e): $C_{22}H_{26}N_8O_8$ (M + 1) calcd 531.1952, found 531.1968.

13. Yield: 13.6%. ^{1}H NMR (CD_3OD, δ): 8.12 (s, 2 H, H2), 6.91 (s, 2 H, H5), 5.93 (d, 2 H, J = 6.4 Hz, H1′), 4.50–3.60 (m, 10 H, H4′, H3′, H2′, H5′). FAB MS (m/e): $C_{22}H_{26}N_8O_8$ calcd 531.1952, found 531.1954.

Reaction of 5-(Chloromercuri)cytidine with *N,N′*-Bis(trifluoroacetyl)cystamine. 5-(Chloromercuri)cytidine (478 mg, 1 mmol), *N,N′*-bis(trifluoroacetyl)cystamine (860 mg, 2.5 mmol), and a 0.1 M solution of Li_2PdCl_4 in methanol (20 mL, 2 mmol) were stirred at ambient temperature overnight. The mixture was then worked up by treating the solution with H_2S until a brown-black precipitate was formed. The solution obtained on gravity filtration was evaporated under reduced pressure to give a solid that was redissolved in a small amount of methanol and coevaporated with about 3 g of silica gel. Column chromatography (silica gel 50 g, mobile phase a to c) gave fractions containing the R_f = 0.30 (solvent c) or R_f = 0.45 (solvent e) material, which were combined and evaporated to give coupled product **7a**. Yield: 10%. mp 226–228 °C dec. ^{1}H NMR (CD_3OD, δ): 8.48 (s, 1 H, H6), 5.88 (d, 1 H, J = 3.0 Hz, H1′), 4.30–3.70 (m, 5 H, sugar ring), 3.43 (t, 2 H, J = 6.5 Hz, H3″), 2.76 (t, 2 H, J = 6.5 Hz, H2″). ^{13}C NMR (CD_3OD, δ): 167.05 (C4), 159.0 (q, J = 41.9 Hz, C5″), 158.1 (C2), 149.6 (C6), 117.38 (q, J = 286.6 Hz, C6″), 99.5 (C5), 91.8 (C1′), 86.3 (C4′), 76.5 (C2′), 70.7 (C3′), 61.8 (C5′), 39.7 (C3″), 34.8 (C2″). FAB MS (m/e): $C_{13}H_{17}F_3O_6N_4S$ (M + 1) calcd 415.0899, found 415.0890. IR (KBr): 3406 vs (br, NH, OH), 1720 s, 1708 s (C=O), 1624 vs (C=O), 1580 s, 1497 s (C=C), 1216 s, 1185 s, 1165 s (C–F), 1114–1051 (C–O).

Reaction of 5-(Chloromercuri)-2′-deoxycytidine with *N,N′*-Bis(trifluoroacetyl)cystamine. 5-(Chloromercuri)-2′-deoxycytidine (462 mg, 1 mmol), *N,N′*-bis(trifluoroacetyl)cystamine (860 mg, 2.5 mmol), and a 0.1 M solution of Li_2PdCl_4 in methanol (20 mL, 2 mmol) were stirred overnight at room temperature, followed by workup with H_2S. A precipitate that formed during workup was removed by gravity filtration. The procedure for purification was similar to that described for compound **7a**. After column chromatography, fractions containing material with R_f = 0.49 (solvent c) or R_f = 0.61 (solvent e) were combined and evaporated to give the coupled product 5-[3-(trifluoroacetamido)-1-thiapropyl]-2′-deoxycytidine (**7b**). Yield: 5%. mp: 208–209 °C dec. ^{1}H NMR (CD_3OD, δ): 8.41 (s, 1 H, H6), 6.20 (t, 1 H, J = 6.3 Hz, H1′), 4.39 (m, 1 H, H4′), 3.95 (m, 1 H, H3′), 3.73–3.86 (m, 2 H, H5′), 3.45 (t, 2 H, J = 6.3 Hz, H3″), 2.76 (t, 2 H, J = 6.3 Hz, H2″), 2.42–2.16 (m, 2 H, H2′). ^{13}C NMR (CD_3OD, δ): 167.3 (C4), 159.0 (q, J = 4.19 Hz, C5″), 157.5 (C2), 149.7 (C6), 117.4 (q, J = 286.6 Hz, C6″), 98.4 (C5), 89.0 (C1′), 87.8 (C4′), 71.7 (C3′), 62.6 (C5′), 42.2 (C2′), 39.7 (C3″), 34.8 (C2″). FAB MS (m/e): $C_{13}H_{17}F_3N_4O_5S$ (M + 1) calcd 399.0950, found 399.0946. IR (KBr): 3510 s (NH), 3432 s (NH), 3343 vs (OH), 3061 m (=CH), 2934 m (CH_2), 1727 vs (C=O), 1650 vs (C=O), 1631 vs (NH), 1586 s, 1497 s (C=C); 1478–1408 (CH_2, CN), 1224 s, 1193 s, 1152 s (C–F).

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Supplementary Material Available: ^{1}H NMR and ^{13}C NMR spectra of compounds **4, 7, 10, 11, 12,** and **13** (19 pages). Ordering information is given on any current masthead page.