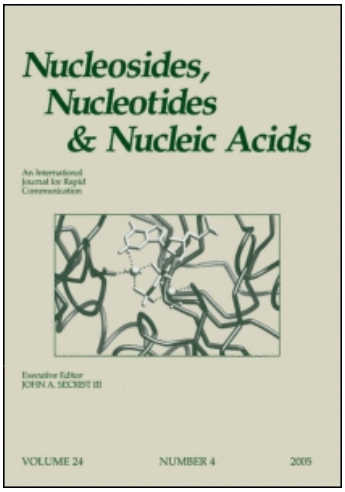

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Ralph J. Cisneros[a]; Louis A. Silks III[a]; Jerome D. Odom[a]; R. Bruce Dunlap[a]
[a] Dept. of Chemistry, University of South Carolina, Columbia, South Carolina

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF VARIOUS SELENENYL AND TELLURENYL-SUBSTITUTED DEOXYURIDINES AND DEOXYURIDYLATES

Ralph J. Cisneros, Louis A. Silks III, Jerome D. Odom*, and R. Bruce Dunlap*
Dept. of Chemistry, University of South Carolina, Columbia, South Carolina 29208

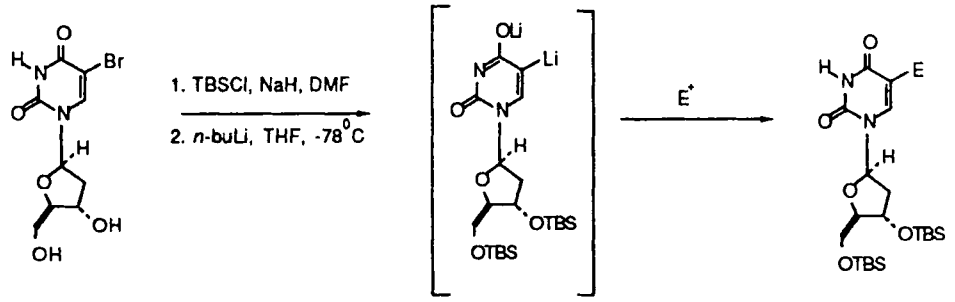
Abstract: 5-Methylselenenyl and 5-phenyltellurenyldeoxyuridines were constructed via the transmetallation of the corresponding 5-bromo-3',5'-bis(dimethyl-*tert*-butyl)silyl deoxyuridine with *n*-butyllithium followed by electrophilic trapping of the anion with the appropriate reagent. These substituted deoxyuridylates have been evaluated as potenital inhibitors of *Lactobacillus casei* thymidylate synthase.

A long range goal of our laboratories is the elucidation of the mechanism of action of thymidylate synthase[1]. The current model of the mechanism envisions the initial step of the reaction to occur by the nucleophilic attack of the active site thiolate anion of Cys^{198} in a Michael sense at C-6 of the non-covalently bound pyrimidine nucleotide[1-5]. This initial step of the mechanism yields a covalently bound enzyme-nucleotide binary complex, which has been characterized by ^{19}F NMR spectroscopy[6] and the trichloroacetic acid (TCA) precipitation assay[7]. The ^{19}F NMR data suggest that the covalent binary complex is in equilibrium with the non-covalent complex. In addition, the observed chemical shift for the covalent binary complex is consistent with a fluorine attached to a fully saturated carbon at C-5. These results require the existence of an enol (or enolate) intermediate. In the presence of the cofactor, this intermediate is converted into the well-documented inhibitory ternary complex[1-5]. The natural substrate, dUMP, and the product, dTMP, have also been shown to form equilibrium complexes with the enzyme in the absence of folates with binding ratios of 0.15 and 0.11, respectively[7]. In contrast, FdUMP exhibits a binding ratio of 0.75 for the covalent binary complex. Evidently, the isosteric replacement of hydrogen with fluorine activates the enone system toward nucleophilic attack and, in addition, stabilizes the proposed intermediate.

In an effort to support the current model of the mechanism, derivatives which could affect the formation and stability of the intermediate, such as selenium and tellurium containing substituents at C-5, were targeted for synthesis and evaluation.

The rationale for these experiments was to obtain compounds whose interaction with the enzyme could be investigated by the TCA assay and NMR spectroscopy. It is a well-known fact that selenium and tellurium atoms stabilize adjacent carbanions[8], and by a judicious choice of groups attached to the chalcogens substituted at C-5, it should be possible to modulate the extent of covalent binding of these dUMP analogs to the enzyme.

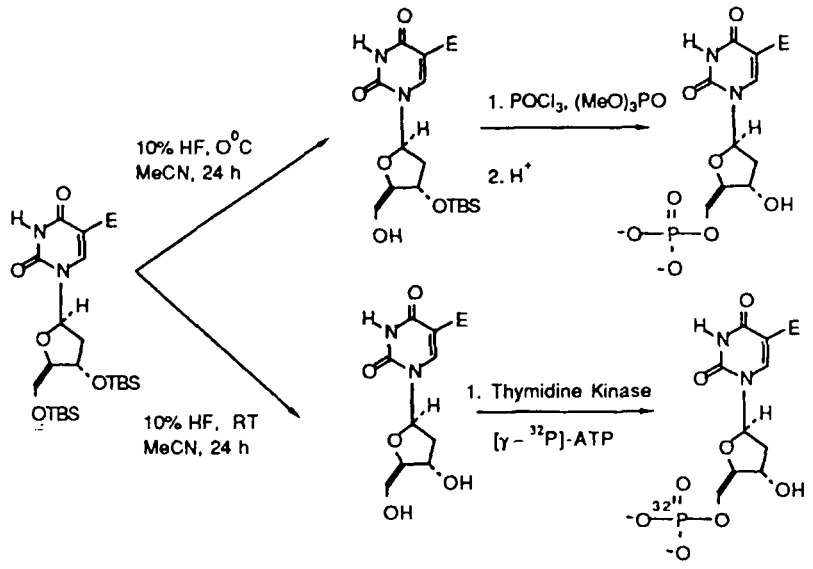
Materials and Methods: Thymidylate synthase was obtained from amethopterin-resistant *Lactobacillus casei* and was purified to homogeneity on a Pharmacia FPLC system by ion-exchange chromatography on a MonoQ column followed by affinity chromatography on a 10-formyl folate column[9]. Thymidine kinase was obtained from *E. coli* HB 101 grown in minimal LB media and was partially purified on a Pharmacia FPLC system by ion-exchange chromatography on a MonoQ column followed by gel filtration on Sephacryl S-300 column. Elemental selenium and tellurium as grey powders (200 mesh), dimethyl diselenide, and dimethyl telluride were obtained from Alfa Products and were used without further purification. 5-Bromo-2'-deoxyuridine and *p*-fluorobromobenzene were supplied by Sigma Chemical Co. *Tert*-butyldimethylchlorosilane was supplied by Petrarch Systems Inc. Sodium hydride (used as an 80% oil dispersion), Silica gel (230-400 mesh) and alkyl- and aryllithiums were obtained from Aldrich Chemical Co. The aromatic ditellurides, diphenyl ditelluride (ØTeTeØ) and bis(*p*-fluorophenyl)ditelluride, were prepared via the addition of the appropriate aryllithium to a suspension of elemental tellurium in tetrahydrofuran, followed by air oxidation[10]. The concentration of alkyllithium reagents in commercial solutions was determined by titration with diphenylacetic acid to the yellow endpoint[11]. Tetrahydrofuran was distilled from sodium/benzophenone prior to use. NMR spectra were recorded as $CDCl_3$ solutions on Bruker AM-300 [^{1}H, ^{77}Se (operating at 57.24 MHz)], Bruker WP-200 (^{125}Te , operating at 68.53 MHz) or IBM NR-80B (^{13}C, operating at 20.11MHz) instruments. ^{125}Te, ^{77}Se, and ^{13}C spectra were obtained employing continuous broad-band noise-modulated proton decoupling at ambient temperature. ^{77}Se chemical shifts were measured with respect to external dimethyldiselenide [60% in $CDCl_3$ (v/v)][12]. A spectral window of several hundred ppm, a pulse angle of 30° and a recycle time of two seconds were employed. ^{125}Te chemical shifts are reported as values in parts per million relative to 60% solutions of dimethyl telluride in $CDCl_3$ (v/v)[12]. Generally, 1000-5000 scans were acquired using a pulse angle of 35° and a recycle time of 0.2 s. ^{13}C chemical shifts are reported in parts per million relative to tetramethylsilane and referenced with respect to internal $CDCl_3$ (δ = 77.0 ppm). Mass spectra were measured on a Finnigan 4021 GC/MS spectrometer.



SCHEME I

Synthesis: The starting material for substitution reactions at C-5 was the 5-bromo-2'-deoxyuridine (Br-dUrd). Using transmetallation technology, it was possible to generate a carbanion at C-5 regioselectively[13]. Prior to transmetallation, the 3'- and 5'-hydroxyl groups of Br-dUrd were protected with the *t*-butyldimethylsilyl (TBS) group. This was effected by treatment of the starting material with five equivalents of *tert*-butyldimethylchlorosilane (TBSCl) in distilled, dry dimethyl formamide (DMF) followed by the slow addition of four equivalents of NaH at room temperature. The reaction was monitored for completion using thin-layer chromatography on silica gel plates (30% diethyl ether/hexanes (v/v)). The crude reaction mixture was taken up in diethyl ether and was washed five times with water followed by brine. The crude reaction product was dried over $MgSO_4$, filtered and volatiles were removed *in vacuo*. The desired product was purified by flash column chromatography (20% diethyl ether/hexanes (v/v), using 230-400 mesh silica gel). The 3',5'-bis(silyl)ether was taken up in THF under anhydrous conditions and chilled to -78 °C. Subsequently, 2.5 equivalents of *n*-butyllithium were added, generating the C-5 carbanion. The resulting carbanion can be trapped by a variety of electrophilic reagents as described in Scheme I[14].

Selective monodeprotection of the 5'-silyl ether can be achieved by the action of 10% HF in acetonitrile at 0 °C over a period of 24 hours in >90% yield. Complete deprotection was effected using the same conditions, with the exception that the reaction was stirred at room temperature. Selective chemical phosphorylation at the 5' position



SCHEME II

was carried out with phosphorus oxychloride in trimethylphosphate[15-16] (Scheme II). Enzymatic phosphorylation at the 5' position was also accomplished using [γ-^{32}P]-ATP and partially purified thymidine kinase obtained from *E. coli*.

Results and Discussion: The fully deprotected nucleosides, 5-selenenylmethyl-2'-deoxyuridine (SeMedUrd), 5-phenyltellurenyl-2'-deoxyuridine (ØTedUrd), and 5-(*p*-fluorophenyl)tellurenyl-2'-deoxyuridine (FØTedUrd), were converted to the corresponding 5'-monophosphates by the action of thymidine kinase in the presence of [γ-^{32}P]-ATP (3000 Ci/mmol). The reaction was monitored for completion by inspection of the autoradiogram obtained from thin layer chromatography of the reaction mixture on PEI-cellulose plates that were developed with 1.25 M LiCl. The [^{32}P]-labeled nucleotides were purified by ion-exchange chromatography on a DEAE-cellulose column using a linear gradient of NH_4HCO_3 from 0.05-1.0 M. The nucleotides were pooled, lyophilized, and dissolved in 1 mL water. Yields for the [^{32}P]-labeled nucleotides ranged from 30-50% (~35 pmol scale).

The ability of each of the [^{32}P]-labeled nucleotides to form covalent bonds with thymidylate synthase was investigated by incubating the enzyme (0.6 μM) with each of

the nucleotides (0.6 nM) in the presence or absence of cofactor (0.7 mM) in 1) 100 mM acetate buffer, pH 5.8, or 2) 100 mM Tris buffer, pH 7.5, both containing 100 mM 2-mercaptoethanol and 50 mM KCl at room temperature. The reactions were quenched after incubating up to 72 hours by the addition of sufficient TCA to achieve a final concentration of 10%. The precipitates were washed five times with 10% TCA, solubilized with 3x125 μL 0.2 N NaOH, and counted on a Beckman LS 7500 liquid scintillation counter[7]. The data are as follows.

These data suggest that little, if any, of the nucleotide analogs is bound covalently to thymidylate synthase under these conditions, regardless of the presence of cofactor. Similarly, autoradiography of the binary and ternary complexes mixtures following SDS-PAGE revealed only a slight indication of the presence of covalent complexes. The existence of non-covalent interactions between thymidylate synthase and each of the nucleotides was not addressed in the studies reported here.

In summary, selenium and tellurium derivatives of dUMP have been made and characterized by ^{1}H, ^{13}C, ^{77}Se, and ^{125}Te NMR. These selenium- and tellurium-containing nucleosides are substrates for *E. coli* thymidine kinase. The mechanistic model for thymidylate synthase predicts that at least two factors, activation of C-6 for nucleophilic attack and stabilization of the transient carbanion at C-5, contribute to the extent of covalent enzyme-nucleotide complex formation. Since the nucleotides evaluated here exhibited only a slight tendency to form covalent binary and ternary complexes with the enzyme, the design of the next group of potential inhibitors in this series should emphasize an increase in the electron withdrawing capacity of the groups attached to the chalcogens at C-5. The presence of these groups should both activate the C-6 position of the nucleotide for enzymic attack and stabilize the incipient carbanion at C-5.

TABLE 1

Results of TCA Assays for Covalent Nucleotide Binding.[a]

Nucleotide	BC[b]	TC[c]	Theoretical[d]
SeMedUMP	1488	4589	500,000
ØTedUMP	4544	5144	500,000
FØTedUMP	5665	6909	500,000

[a]The data are provided in terms of dpm.
[b]Covalent enzyme-nucleotide binary complex.
[c]Covalent enzyme-nucleotide ternary complex formed in the presence of cofactor.
[d]Total dpms expected if all added nucleotide was bound covalently.

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