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A NEW APPROACH TO THYMIDYLATE SYNTHETASE INHIBITORS

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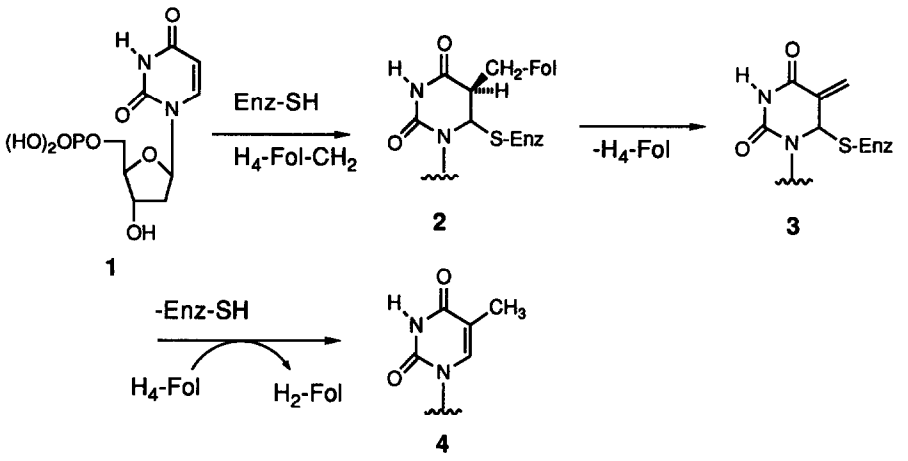
Abstract *A new approach to the design of thymidylate synthase inhibitors for cancer chemotherapy is described Several uracil and 2'-deoxyuridine derivatives bearing vinylsulfoxide moieties at C-5 were prepared The two methods described involve a Pd-catalyzed coupling utilizing suitably substituted organostannanes, and a stepwise route involving the regioselective addition of sulfenyl chlorides to 5-vinyluracil and its corresponding nucleosides*

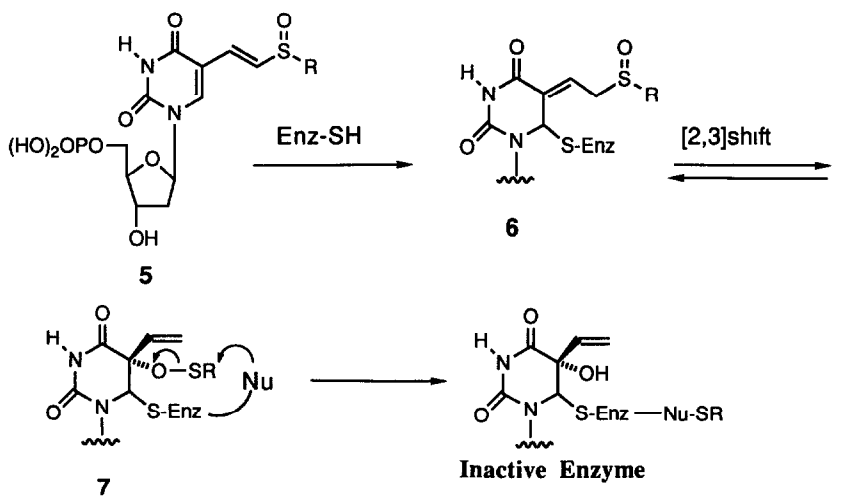
Thymidylate Synthetase (TS) is one of the key enzymes involved in DNA synthesis It catalyzes the reductive methylation of 2'-deoxyuridylate **1** to thymidylate **4** (Scheme 1) The methyl group is provided by the cofactor 5,10-methylene-5,6,7,8-tetrahydrofolate (indicated as H_4-Fol-CH_2), which is oxidized to 7, 8-dihydrofolate (H_2-Fol) in the process [1] The first step of the catalytic process involves nucleophilic addition of the enzyme (by way of a cysteine residue), with formation of ternary complex **2** Since DNA synthesis is crucial to rapid cell replication, TS has traditionally been a favorite target in anti-cancer drug design [1,2] Indeed the clinically established agent 5-fluorouracil (5-FU) [3] is thought to act as a metabolic precursor of the corresponding nucleotide (FdUMP), which forms a very stable ternary complex with the enzyme and the cofactor, [4] and therefore inactivates TS Although other targets have been proposed for 5-FU, there is evidence that TS inactivation is the major mechanism of action [5,6] The strategy we propose involves the design of new suicide inhibitors [7] More specifically, we intended to exploit the use of masked allylic sulfoxides, first introduced by Firestone and Walsh, [8] as shown in Scheme 2

SCHEME 1



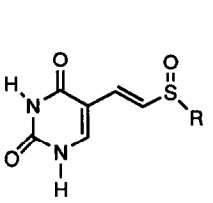
The inhibitor **5** would initially be processed by the enzyme in the same way as its natural substrate, literature precedents demonstrate that the enzyme is tolerant of functionality at C-5 [1] Michael addition should also be favored by the strongly electron-withdrawing vinyl sulfoxide moiety at C-5 [1] Subsequent protonation should produce allylic sulfoxide **6** , in preference to the corresponding vinyl analog, due to thermodynamic reasons

SCHEME 2

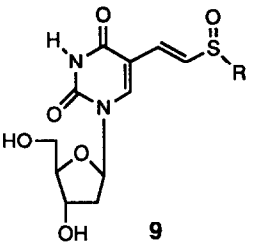


Compound **6** is in equilibrium with the corresponding sulfenate **7**, as shown by Mislow, [9] and the highly electrophilic **7** may react with a nucleophile within the active site, deactivating the enzyme (Scheme 2). However, since a highly ionizable compound such as **5** would probably be poorly transported through the membrane of tumor cells, we decided to prepare the corresponding base **8** and its nucleoside derivative **9** as

potential chemotherapeutic agents *In vivo*, **8** and **9** may be interconverted by the intracellular salvage enzyme thymidine phosphorylase Thymidine kinase may then effect phosphorylation, producing active species **5** [1]



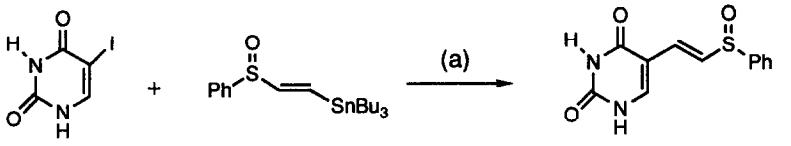
8



9

Our preparation of the first member of this class of compounds followed the recently reported preparation of 5-substituted uracils via the Stille reaction (Eq 1) [10] Once again, the use of tri(2-furyl)phosphine (TFP) proved crucial to the obtention of **12** in high yields

(1)



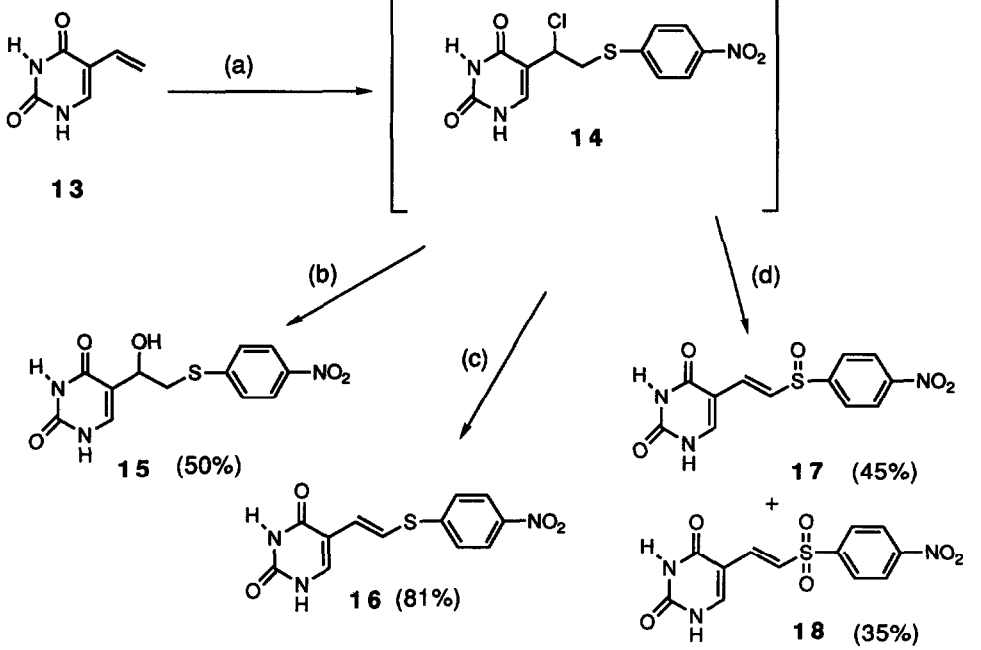
10 **11** **12**

a) Pd_2dba_3, TFP, NMP, rt, 78-88%

Compound **12** showed no measurable toxicity against a panel of human tumor cells (including colon carcinoma and leukemia) One of the possible reasons for this observation may be the lack of electron-withdrawing substituents on the sulfoxide moiety It has been shown that for best results the inhibitor should be a para-nitrophenyl sulfoxide [8] The difficulty encountered in making other sulfoxide-containing vinyl stannanes by traditional methods [11] led us to explore new chemistry, and eventually discover a more convenient route to our proposed inhibitors

It is known that addition of p-nitrophenylsulfenyl chloride to terminal olefins yields the anti-Markovnikoff product as the kinetic adduct, and that equilibration to the thermodynamic product is very difficult [8] In our case, this would therefore lead to the undesired mode of addition However, when we treated 5-vinyluracil [10] with p-nitrobenzenesulfenyl chloride in THF at room temperature (Scheme 3) a rapid reaction ensued to yield a product that was chromatographically unstable Examination of an aliquot by ^{1}H-NMR spectroscopy, however, showed complete conversion to one species, tentatively identified as **14** This was then confirmed by quenching the mixture with bicarbonate, which produced **15** as the major product Treatment with triethylamine yielded instead vinylsulfide **16** Finally, oxidation of **14** with a slight excess of meta-chloroperbenzoic acid, followed by triethylamine, gave the desired sulfoxide **17**, together with the overoxidation product **18** The sulfide and sulfone analogs of **17** are control compounds if bioactivity is only due to our proposed mechanism, the sulfoxide, but not the sulfide or the sulfone, should display cytotoxicity

SCHEME 3

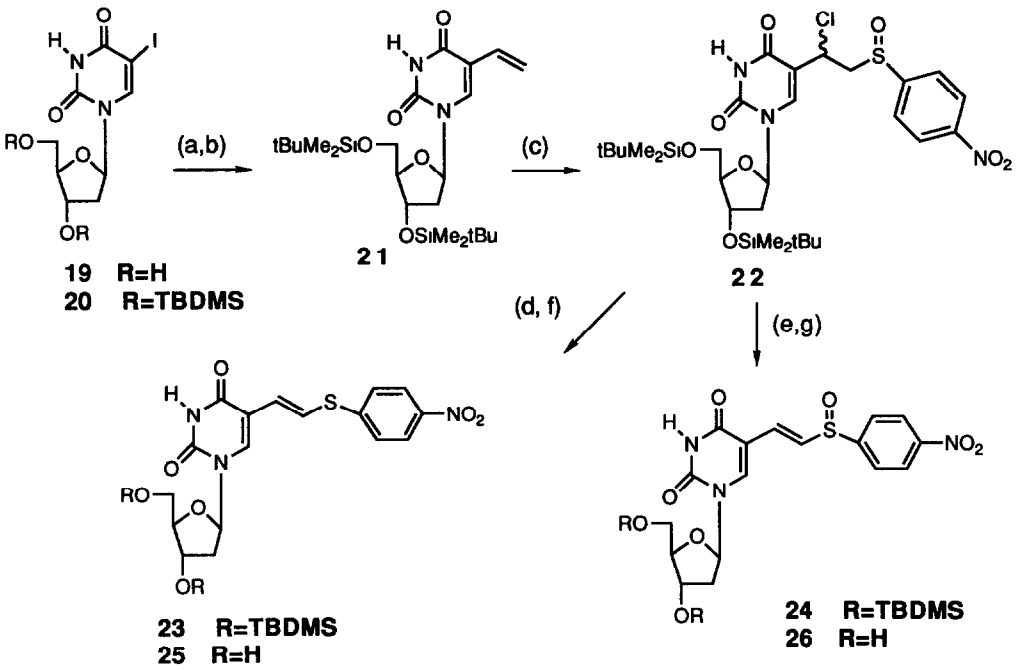


a) pNO2-C6H4-SCl,THF,rt. b) aq NaHCO3 c) NEt3, rt. d) m-CPBA, then NEt3

Evidently, in this particular case the regiochemistry of the addition favors the production of the Markovnikoff adduct This may be the result of a reversed kinetic preference, as N(1) of the uracil ring should strongly affect the electron density in the vinyl group at C-5 Alternatively, kinetic preference may be unchanged, and formation of **14** may simply be the result of ready equilibration This is supported by the observation that **14** is very unstable and easily solvolyzes, demonstrating that N(1) in the uracil readily supports a carbonium ion at the C(5') position

In any event, the same method worked well in the nucleoside series (Scheme 4) Thus, 2'-deoxy-5-iodouridine was smoothly silylated, and coupled with vinyltributyltin according to the modified Stille protocol Addition of para-nitrobenzenesulfenyl chloride gave rise to a mixture of two adducts, as evidenced by ^{1}H-NMR. That these were merely diastereomers was shown by elimination with triethylamine, which cleanly provided only the terminally substituted aryl sulfide Oxidation followed by elimination provided instead compound **24** as a 1 1 diastereomeric mixture of the two sulfoxides Desilylation of **23** with fluoride gave **25** but produced a rather complex mixture when applied to **24** The use of Dowex 50W-X8, [12] however, provided **26** in good yield Compounds **12, 16, 17, 18, 25** and **26** displayed no useful cytotoxicity (IC_{50} > 10μg/ml) vs our panel of tumor cells This may due to lack of phosphorylation of **26** by intracellular kinases, or to unfavorable catabolism, rather than a failure of the phosphorylated substrate to interact with TS according to the postulated mechanism In any event, the targeting of TS with our sulfoxide-containing uracil derivatives appears very difficult at this stage

SCHEME 4



(a)TBSCl,Imidazole,NMP,rt,92% (b)CH_2=CHSnBu3,Pd_2dba3,TFP,NMP,rt,72% (c)pNO_2-C_6H_4-SCl,THF,rt (d)NEt3,rt,69% (e) m-CPBA, then NEt3, 51% (f) NBu_4F, THF, 87% (g) Dowex, MeOH, 72%

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EXPERIMENTAL

General experimental protocols are as previously described [13] Proton Nuclear Magnetic Resonance experiments were run at 300 MHz on a Bruker AC-300 spectrometer 5-Iodouracil and 2'-deoxy-5-iodouridine were obtained from Sigma and used directly Tri(2-furyl) phosphine was prepared by the method of Allen [14] All reactions were monitored by thin-layer chromatography (SiO_2) except for the Pd-promoted couplings, which were monitored by reversed-phase HPLC [13]

(E)-2-(Tributylstannyl)ethenylphenylsulfoxide, 11.

(E)-2-(Tributylstannyl)ethenylphenylsulfide , which was prepared according to Keck in 36% yield [11], (1 425 g, 3 351 mmol) in anhydrous dichloromethane (5 mL) at -78°C was treated with m-chloroperbenzoic acid (85%, 573 mg, 2 822 mmol) in 5 batches over 10 min The temperature was allowed to reach 0°C over 1h, then work-up with aqueous sodium bicarbonate (5%, 100 mL) and ethyl acetate (100 mL) gave a crude product that was purified by silica chromatography (10% ethyl acetate in hexane), to yield a colorless oil,

402mg (32% yield) NMR ($CDCl_3$) δ 7 58-7 40 (m, 5H), 7 34 (d, J= 17 5 Hz, 1H), 6 54 (d, J= 17 5 Hz, 1H), 1 60-0 75 (m, 27H)

5-(2-Phenylsulfinyl)ethenyluracil, 12

5-Iodouracil (49 mg, 0 205 mmol) was dissolved in anhydrous N-methylpyrrolidinone (NMP, 2 mL) and treated with tri(2-furyl)phosphine (3 0 mg, 0 0129 mmol) and tris(dibenzylideneacetonyl)bis-palladium (3 0 mg, 0 0065 mmol Pd) When the red solution had turned pale yellow, stannane **11** (76 mg, 0 1458 mmol) was added in NMP solution (1 mL) The solution was stirred under argon for 16 h The NMP was evaporated *in vacuo*, and the residue partitioned between hexane and acetonitrile The acetonitrile layer was evaporated and the residue chromatographed (ethyl acetate followed by 2-3% methanol/ EtOAc) to elute **12**, which was isolated as a tan solid, m p 221-222°C (dec)

NMR (DMSO-d_6) δ 11 5-11 3 (br s, 2H), 7 95 (s, 1H), 7 63-7 36 (m, 5H), 7 39 (d, J=15 2 Hz, 1H), 7 01 (d, J=15 2 Hz, 1H) HRMS Calcd for $C_{12}H_{10}N_2O_3S$ 262 0412, found 262 0408

5-[(1-Hydroxy-2-(4-nitrophenylthio)]ethyluracil, 15.

5-Vinyluracil [10] (62 mg, 0 449 mmol) was suspended in dry THF (2 mL) and p-nitrophenylsulfenyl chloride (Aldrich, 86 mg, 0 454 mmol) was added by syringe in THF solution (2 5 mL) The slurry turned into a solution within 5 min, and after 2 h at RT, the reaction was worked up by adding aqueous sodium bicarbonate (10 mL), stirring for 10 min, and partitioning between water and ethyl acetate The organic layer was further washed with water and brine, then dried with sodium sulfate Silica gel chromatography (10% methanol in dichloromethane) gave a yellow amorphous solid, 70 mg (50% yield)

NMR (DMSO-d_6) δ 11 2 (s, 1H), 10 82 (br d, 1H), 8 08 (d, J=8 9 Hz, 2H), 7 57 (d, J=8 9 Hz, 2H), 5 62 (d, J=5 1 Hz, 1H), 4 64 (m, 1H), 3 47 (dd, J=13 5 Hz, J'= 3 3 Hz, 1H), 3 15 (dd, J=13 5 Hz, J'= 7 6 Hz, 1H)

HRMS Calcd for $C_{12}H_{12}N_3O_5S$ (MH^+) 310 0498, found 310 0491

5-[2-(4-Nitrophenylthio)]ethenyluracil, 16.

5-Vinyluracil (82 mg, 0 5937 mmol) was suspended in dry THF (2 mL) and treated as above with p-nitrophenylsulfenyl chloride (148 mg, 0 7805 mmol) in THF (1 mL) After 1 h at RT triethylamine (0 085 mL, 0 610 mmol) was added neat, and stirring was continued for 16h Then methanol (5 mL) was added, and the resulting precipitate was stirred at 0°C for 1h, filtered, and washed with cold methanol

The sample of **16** thus obtained weighed 141 mg (81% yield), m p 298-299°C (dec)

NMR (DMSO-d_6) δ 11 5 (br m, 2H), 8 18 (d, J=8 9 Hz, 2H), 7 78 (d, 1H), 7 48 (d, J= 8 9 Hz, 2H), 7 39 (d, J=15 5 Hz, 1H), 6 73 (d, J=15 5 Hz, 1H) HRMS Calcd for $C_{12}H_{10}N_3O_4S$ (MH^+) 292 0392, found 292 0386

5-[2-(4-Nitrophenylsulfinyl)]ethenyluracil, 17, and 5-[2-(4-nitrophenylsulfonyl)]ethenyluracil, 18.

5-Vinyluracil (85 mg, 0 615 mmol) in dry THF (3 mL) was treated with p-nitrobenzenesulfenyl chloride (116 4 mg, 0 615 mmol) in THF (1 9 mL) as above After 1h at RT, the solution was cooled to -78°C and m-chloroperbenzoic acid (85%, 129 mg, 0 625 mmol) in THF (3 mL) was added by syringe The mixture was slowly allowed to reach 0° C (1 h), and triethylamine (0 170 mL, 1 234 mmol) was added neat After 1 h at 0°C, the slurry was stirred at RT for 1h The suspension was evaporated to dryness, and the residue chromatographed Sulfone **18** eluted first (2% methanol in dichloromethane), and was isolated as an amorphous colorless solid (67 5 mg, 35% yield), while elution with 5% methanol gave sulfoxide **17** (85 mg, 45% yield) as a tan solid, m p 230-232°C (dec) Compound **17** gave the following data

NMR (DMSO-d6) δ 11 5 (s, 1H), 11 4 (s, 1H), 8 39 (d, J=8 9Hz, 2H), 7 99 (br d, 1H), 7 90 (d, J=8 9 Hz, 2H), 7 46 (d, J=15 1 Hz, 1H), 7 08 (d, J=15 1 Hz, 1H) HRMS Calcd for $C_{12}H_{10}N_3O_5S$ (MH^+) 308 0341, found 308 0337 Compound **18** gave the following data

NMR (DMSO-d6) δ 11 8 (s, 1H), 11 5 (s, 1H), 8 41 (d, J=8 9Hz, 2H), 8 20 (br d, 1H), 8.10 (d, J=8 9 Hz, 2H), 7 50 (s, 2H) HRMS Calcd for $C_{12}H_{10}N_3O_6S$ (MH^+) 324 0290, found 324 0280

1-(2-Deoxy-3,5-di-tertbutyldimethylsilyl-β-D-ribofuranosyl)-5-ethenyluracil, 21.

2'-Deoxy-5-iodouridine (2 348 g, 6 631 mmol) was dissolved in dry NMP (20 mL) and treated with imidazole (2 250 g, 0 03305 mol) and t-butyldimethylsilyl chloride (2 501 g, 0 01659 mmol) for 2h at RT Work-up with water and ethyl acetate was followed by silica gel chromatography (20% ethyl acetate in hexane) gave **20** as a foam (3 569 g, 92.4% yield)

NMR ($CDCl_3$) δ 8 50 (br s, 1H), 8 02 (s, 1H), 6 22 (m, 1H), 4 32 (m, 1H), 3 91 (m, 1H), 3 83 (br d, J=9 Hz, 1H), 3 68 (br d, J= 9 Hz, 1H), 2 23 (m, 1H), 1 88 (m, 1H), 0 88 (s, 9H), 0 81 (s, 9H), 0 10 (s, 6H), 0 02 (s, 6H)

Compound **20** (1 092 g, 1 874 mmol) was dissolved in dry NMP (7 mL) and treated with tri(2-furyl)phosphine (17 4 mg, 0 0750 mmol), tris(dibenzylideneacetonyl)bis-palladium (17 2 mg , 0 0376 mmol) followed by vinyltributyltin (0 60 mL, 2 061 mmol) The mixture was stirred at RT under argon for 14h Work-up by partitioning between water and ethyl acetate was followed by further washing the organic layer with three volumes of water Drying and evaporation gave a crude product, that was redissolved in acetonitrile and washed with three volumes of hexane Evaporation and silica chromatography (20% ethyl acetate in hexane) gave **21** as a colorless foam (653 mg, 72% yield)

NMR ($CDCl_3$) δ 8 10 (br s, 1H), 7 61 (s, 1H), 6 30-6 15 (m, 2H), 5 92 (br d, J= 18 Hz, 1H), 5 17 (br d, J=12 Hz, 1H), 4 33 (m, 1H), 3 89 (m, 1H), 3 75 (AB q, 2H) , 2 2 (m, 1H), 1 92 (m, 1H), 0 84 (s, 9H), 0 81 (s, 9H), 0 05 (s, 6H) 0 01 (s, 6H) HRMS Calcd for $C_{23}H_{43}N_2O_5Si$ (MH^+) 483 2711, found 483 2705

1-(2-Deoxy-β-D-ribofuranosyl)-5-[2-(4-nitrophenylthio)]ethenyluracil, 25.

5-Vinyluridine derivative **21** (225 mg, 0 466 mmol) was dissolved in dry THF (3 mL) and p-nitrobenzenesulfenyl chloride (97 mg, 0 513 mmol) was added as a THF (1 2 mL) solution at 0°C After 30 min at 0°C and 1h at RT, triethylamine (0 130 mL, 0 925 mmol) was added neat, and the solution was stirred overnight Dilution with ethyl acetate and washing with three portions of water was followed by drying, and silica chromatography Elution with 25% ethyl acetate in hexane gave 204 mg (69%) of **23** as a yellow foam

NMR ($CDCl_3$) δ 8 30 (br s, 1H), 8 08 (d, J=8 9 Hz, 2H), 7 71 (s, 1H), 7 52 (d, J=15 5 Hz, 1H), 7 33 (d, J=8 9 Hz, 2H), 6 48 (d, J=15 5 Hz, 1H), 6 23 (m, 1H), 4 32 (m, 1H), 3 75 (AB q, 2H), 2 28 (m, 1H) , 1 95 (m, 1H), 0 83 (s, 9H), 0 81 (s, 9H), 0 05 (s, 6H), 0 02 (s, 6H)

This foam (200 mg, 0 3145 mmol) was dissolved in dry THF (5 mL) and treated with tetrabutylammonium fluoride (1M in THF, 0 6 mL) for 3h at RT Evaporation and chromatography (silica, 5-10% methanol in dichloromethane) gave **25** as a solid Recrystallization from little methanol gave a yellow powder, 112 mg (87%), m p 225-227°C (dec) NMR (DMSO-d6) δ 11 6 (s, 1H), 8 19 (d, J=9 Hz, 2H), 8 18 (s, 1H), 7 49 (d, J=9 Hz, 2H), 7 36 (d, J=15 2 Hz, 1H), 6 75 (d, J=15 2 Hz, 1H), 6 14 (m, 1H), 5 25 (d, J=4 2 Hz, 1H), 5 11 (br t, 1H), 4 24 (m, 1H), 3 78 (m, 1H), 3 59 (m, 2H), 2 15 (m, 2H) HRMS Calcd for $C_{17}H_{18}N_3O_7S$ (MH^+) 408 0865, found 408 0848

1-(2-Deoxy-β-D-ribofuranosyl)-5-[2-(4-nitrophenylsulfinyl)]ethenyluracil, 26.

5-Vinyluridine derivative **21** (356.6 mg, 0 739mmol) was dissolved in dry THF (5 mL) and p-nitrobenzenesulfenyl chloride (153 8 mg, 0 812 mmol) was added as a THF (1 9 mL) solution at 0°C After 30 min at 0°C and 1h at RT, the solution was cooled to -50°C, and meta-chloroperbenzoic acid (150 mg, 0 739 mmol) in THF (3 mL) was added The temperature was allowed to reach 25°C over 90 min Triethylamine (0 20 mL, 1 478 mmol) was added neat, and the solution was stirred for 30 min Addition of aqueous 5% sodium bicarbonate and ethyl acetate was followed by separation, and washing the organics with more water The crude product was chromatographed (50% ethyl acetate/hexane) to give a foam (249 mg, 51 7% yield) NMR ($CDCl_3$) δ 8.28 (d, J=9 0 Hz, 2H) 8 03 (s, 3H) 7 71 (d, J=9 Hz, 2H) 7 54 (d, J=15 2 Hz, 1H) 6 82 (d, J=15 2 Hz, 1H) 6 18 (m, 1H) 4 32 (m, 1H) 3 95 (m, 1H) 3 77 (AB q, 2H) 2 28 (m, 1H) 1 93 (m, 1H) 0 9-0 8 (m, 18H) 0 15-0 00 (m, 12 H)

This foam (249 mg, 0 382 mmol) in methanol (10 mL) was stirred at RT for 72 h with methanol-washed Dowex 50X8-100 resin (6 g) Filtration, rinsing with more methanol evaporation and silica chromatography (10% methanol in dichloromethane), followed by recrystallization from methanol, gave **26** as a pale yellow solid (1 1 mixture of diastereomers), 118 mg (72%) having m p 110-141°C (slow dec) NMR (DMSO-d_6) δ 11 6 (s, 1H), 8 39 (d, J=8 9 Hz, 2H), 8 33 (s, 1H), 7 92 (d, J=8 9 Hz, 2H), 7 47 (2 d's, J=15 2 Hz, 0 5H each), 7 04 (d, J=15 2 Hz, 1H), 6 10 (m, 1H), 5 25 (d, J=4 3 Hz, 1H), 5 13 (br t, 1H), 4.23 (m, 1H) , 3 77 (m, 1H), 3 58 (m, 2H), 2 14 (m, 2H) HRMS Calcd for $C_{17}H_{18}N_3O_8S$ 424 0815, found 424 0806

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