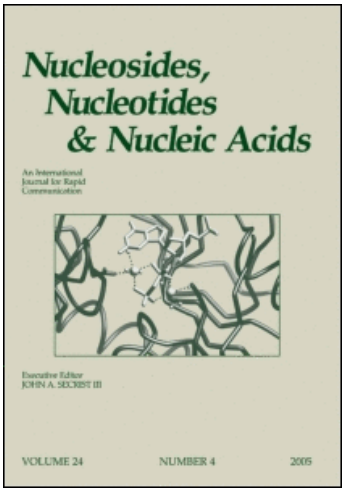

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High-Yield Regioselective Thiation of Biologically Important Pyrimidinones, Dihydropyrimidinones and Their Ribo, 2'-Deoxyribo and 2', 3'-Dideoxyribo Nucleosides

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HIGH-YIELD REGIOSELECTIVE THIATION OF BIOLOGICALLY IMPORTANT PYRIMIDINONES, DIHYDROPYRIMIDINONES AND THEIR RIBO, 2'-DEOXYRIBO AND 2',3'-DIDEOXYRIBO NUCLEOSIDES.

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ABSTRACT. Convenient and high-yield regioselective thiation procedures based on the use of the Lawesson reagent in different solvents, are described for conversion of the 2- and 4-keto, and 2,4-diketo pyrimidines to the corresponding 2(4)-thio, and 2,4-dithio, derivatives. This method is applicable to thiation of the 4-keto groups of 5,6-dihydropyrimidinones and pyrimidine nucleosides. The mild reaction conditions employed are such that it is the method of choice for compounds with labile glycosidic bonds, such as 5,6-dihydropyrimidine nucleosides and the 2',3'-dideoxynucleosides currently of interest as antiretroviral, including anti-HIV, agents.

INTRODUCTION

Thiated pyrimidinones, and their nucleosides and nucleotides, are of considerable biological importance. They are components of the tRNA of various micro-organisms,[1,2] yeasts[3] and mammalian cells[4] as a result of post-transcriptional modifications at the level of precursor tRNA, and play a significant role in translation and its control.[1] Thiated nucleosides exhibit anti-neoplastic properties, e. g. 4-thiouridine selectively inhibits the growth of Ehrlich ascites and mouse L1210 leukemia cells,[5] and 5-fluoro-4-thio-2'-deoxyuridine inhibits the growth of L1210 cells and various hematopoietic human leukemia cells.[6] The nucleotides

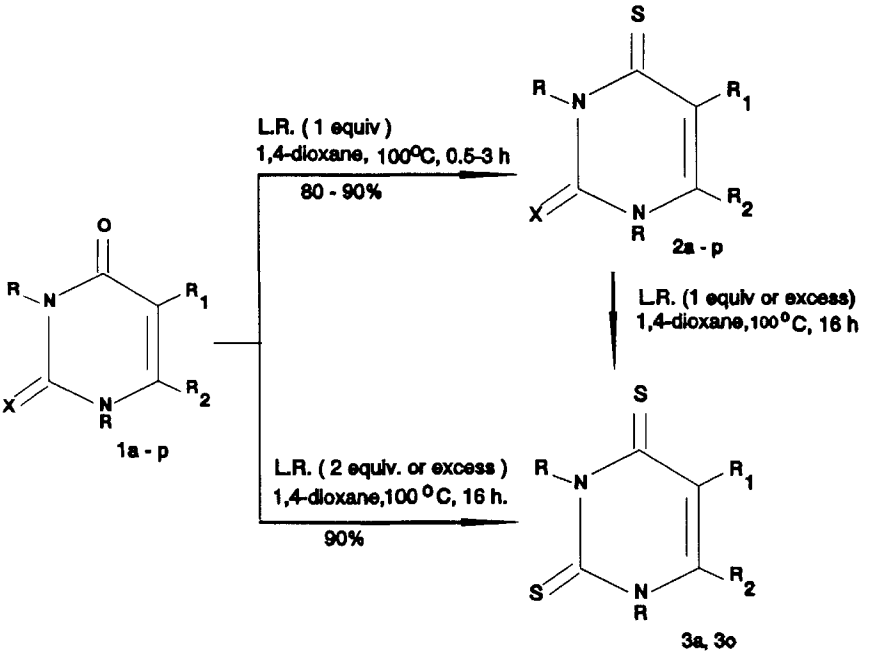
5-fluoro-4-thio-dUMP[7] and 5-fluoro-2-thio-dUMP[8] are potent inhibitors of thymidylate synthase, a target enzyme in tumor chemotherapy. It was recently shown that 2′,3′-dideoxy-3′-fluoro-4-thiothymidine[9] is a potent and selective inhibitor of the retrovirus HIV, the presumed causative agent of AIDS, but the synthesis of this nucleoside was not reported.

Thiation of pyrimidinones and their nucleosides has hitherto been based largely on the use of P_4S_{10} in high-boiling point solvents or in pyridine. The use of refluxing pyridine[10,11] or diglyme-$NaHCO_3$[12] at 110°C leads to thiation of the 4-keto group of 2,4-pyrimidinones and their nucleosides,[10] and in refluxing tetraline[13] or sulfolane[14] to the 2,4-dithio-pyrimidines. Dithiation of pyrimidine ribonucleosides at elevated temperatures in 35% yield has been reported[15], but not hitherto confirmed, and in any event is not applicable to 2′-deoxy- and 2′,3′-dideoxyribo-nucleosides. The more potent Lawesson thiation reagent[16] has received less attention and, where employed, led to 4-thio derivatives.[17,18]

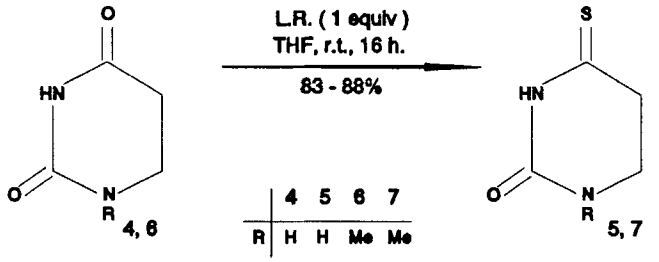
RESULTS AND DISCUSSION

Thiation of pyrimidinones

Our earlier applications of the Lawesson reagent[7,8] pointed to the utility of anhydrous 1,4-dioxane as the optimal solvent. Therefore a systematic study on the thiation of pyrimidinones and their nucleosides with the use of this procedure was undertaken. Under reflux in anhydrous 1,4-dioxane, reaction of each of a series of 5- and 6-substituted, and 5,6-disubstituted, uracils (**1a - 1p**, Scheme 1) in the presence of an equimolar amount of the reagent resulted in complete conversion (monitored by TLC) to the corresponding 4-thio derivatives (**2a - 2p**) independently of the electronic properties and the position of the substituents. The course of the reaction was similar for 2- and 2,6-substituted uracils: isocytosine (**10**) and 6-methyl-2-thiouracil (**1p**).



1,2	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	3a	3o
R	H	H	H	H	H	H	H	H	H	H	H	Me	Me	Me	H	H	H	H
R_1	F	H	H	H	H	F	Me	Cl	H	H	F	H	F	Me	Me	H	F	Me
R_2	H	Me	Et	Pr	i-Pr	Me	Me	Me	CF_3	CH_2Cl	CH_2F	H	H	H	H	Me	H	H
X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	S	–	–



	4	5	6	7
R	H	H	Me	Me

SCHEME 1

Thiation of the 2-keto group of the 4-substituted uracils 2a, 2o and 8 proceeded less readily, but also was not dependent on the nature of the substituent. However, prolongation of the reaction time in the presence of two or more molar equivalents of the Lawesson reagent led to the desired 2,4-dithio derivatives 3a, 3o and the 2-thio derivative 9 in good yields (80 - 90%). To our knowledge this is the first example of successful thiation of the 2-keto group in uracil and cytosine with the use of Lawesson reagent. Previous attempts to thiate the 2-keto group of 4-thiouracil with the same reagent in anhydrous hexamethylphosphoric triamide or pyridine were unsuccessful.[17]

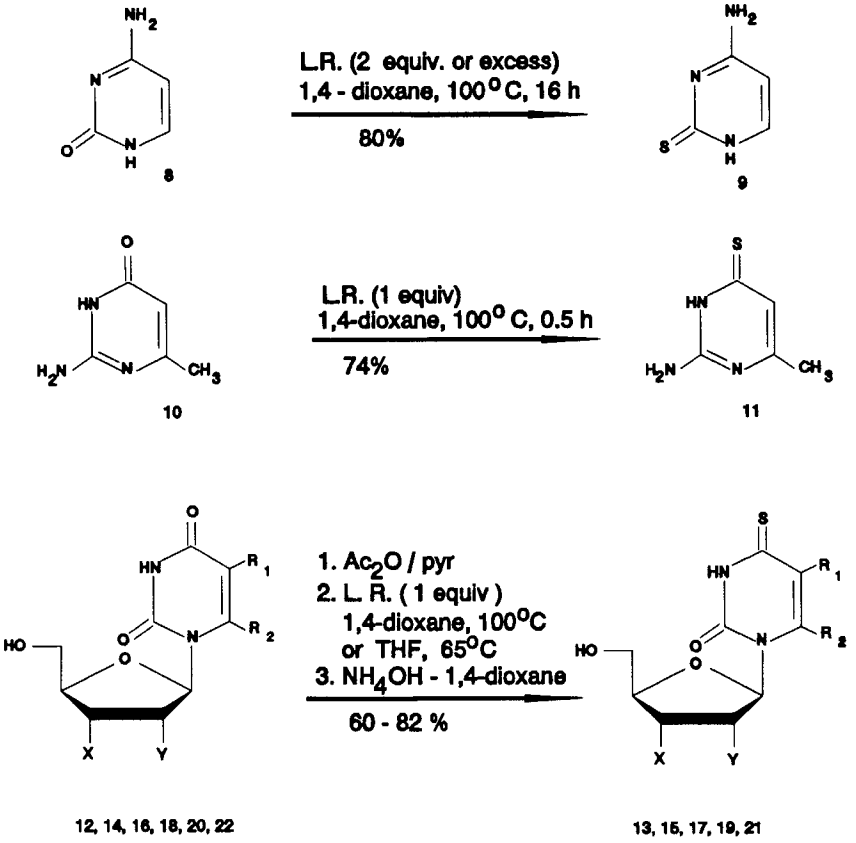
1,3-Dimethyluracil (1l), 1,3-dimethyl-5-fluorouracil (1m) and 1,3,5-trimethyluracil (1n) were readily thiated at C(4). But even a large excess of the reagent and long reaction time left the 2-keto group intact.

Thiation of 5,6-dihydrouracils

A previous report describing the thiation of amides and lactams in tetrahydrofuran (THF) at room temperature[19] prompted us to apply this procedure initially to thiation of 5,6-dihydrouracils. The Lawesson reagent readily dissolved in the more polar THF (relative to dioxane) at room temperature to give the dissociated electrophilic thiating agent $AnPS_2$.[20a] Addition of 5,6-dihydrouracil (4) or its 1-methyl derivative (6, which may be regarded as a counterpart of 5,6-dihydrouridine, a minor component of tRNA) at room temperature led to their regioselective thiation to the corresponding 4-thio derivatives 5 and 7 in high yields (83-88%). In previous report thiation of 4 with P_4S_{10} in refluxing 1,4-dioxane gave only 47% yield of 5.[19] Thiation of 2-keto group of 5,6-dihydrouracils even with a large excess of Lawesson reagent in refluxing THF or in 1,4-dioxane does not occur.

Thiation of pyrimidine nucleosides

The potency of the Lawesson reagent requires protection of the sugar hydroxyls. Hence our procedure



	12	13	14	15	16	17	18	19	20	21	22
R_1	H	H	Et	Et	Me	Me	Me	Me	F	F	Me
R_2	Me	Me	H	H	H	H	H	H	H	H	H
X	OH	OH	OH	OH	OH	OH	F	F	F	F	N_3
Y	OH	OH	OH	OH	H	H	H	H	H	H	H

SCHEME 2

involved acetylation, followed by thiation and removal of acetyl groups, in a "one-pot" synthesis. The reaction was conducted in refluxing 1,4-dioxane, or in tetrahydrofuran, and applied to three classes of nucleosides containing uracil analogues as the aglycon.

Thiation of 6-methyluridine (12) and 5-ethyluridine (14) proceeded smoothly to yield the 4-thio nucleosides 13

TABLE 1. Spectral Data of Thiated Pyrimidinones, 5,6-dihydropyrimidinones and Pyrimidine Nucleosides

Product	UV: λ_{max} [nm](ε) pH 2[a]	pH 7[b]	pH 12[c]	Molecular Formula	MS, m/e Exact Mass Found (Calc)
2a	236(2600) 269(2100) 337(15160)	349(13050)	264 (3900) 325 (9780)	$C_4H_3FN_2O$	145.99501[d] (145.995041)
2b	327(23400)	328(22900)	330(22200)	$C_5H_6N_2OS$	142.02008[d] (142.020111)
2c	246(3770) 328(23600)	247 (2850) 329(22900)	332(20700)	$C_6H_8N_2OS$	156.03573[d] (156.035751)
2d	246(3770) 328(23600)	247 (2850) 329(22900)	332(20700)	$C_7H_{10}N_2OS$	170.05138[d] (170.051407)
2e	255(3290) 333(18900)	330(20800)	332(20350)	$C_7H_{10}N_2OS$[f]	170.05138[d] (170.051407)
2f	236 (2700) 269 (2200) 337(15300)	349(13000)	264 (3800) 325 (9600)	$C_5H_5FN_2OS$[f]	160.010066[d] (160.010681)
2g	337(19350)	337(19700)	227 (7500) 340(18200)	$C_6H_8N_2OS$[f]	156.035730[d] (156.035751)
2h	242 (5100) 324(11300)	240 (5900) 346(10600)	326 (6800)	$C_5H_5ClN_2OS$[f]	175.98111[d] (175.98114)
2i	246 (3050) 331(13850)	246 (3800) 350(17300)	323(11000)	$C_5H_3F_3N_2OS$[f]	195.99182[d] (195.991852)
2j	327(23400)	247 (2800) 329(22900)	330(22000)	$C_5H_5ClN_2OS$[f]	175.97941[d] (175.98114)
2k	236 (2700) 269 (2200) 337(15300)	349(13000)	264 (3800) 325 (9650)	$C_5H_4F_2N_2OS$[f]	178.00123[d] (178.001266)
2l	242 (3500) 327(19100)	246 (3700) 327(19700)	326(19200)	$C_6H_8N_2OS$	156.03573[d] (156.035751)
2m	238 (3200) 272 (3300) 337(18900)	236 (3400) 272 (3400) 337(19500)	237 (3900) 273 (3700) 337(19000)	$C_6H_7FN_2OS$	174.0263[d] (174.026337)
2n	238 (4100) 332(19950)	239 (2800) 332(20500)	238 (4300) 333(19800)	$C_7H_{10}N_2OS$	170.05138[d] (170.051407)

and 15 in high yield (82 and 76% respectively) (Scheme 2). Results were similar for thymidine (16). With 2′,3′-dideoxy-3′-fluorothymidine (18), the most potent nucleoside inhibitor of HIV, the reaction yielded the 4-thio analogue 19, a potent inhibitor of HIV with lower cytotoxicity than 18, albeit with lower yield (60%). The same prevailed for conversion of 2′,3′-dideoxy-3′,5′-difluorouridine (20) to

TABLE 1. Continuation

2p	244(10600) 279 (7800) 336(12900)	254(12900) 312(10900)	255(13450) 311(11400)	$C_5H_6N_2S_2$	157.99654[d] (157.99724)
2o	236 (3700) 333(17100)	235 (4100) 333(17100)	232 (7000) 343(17550)	$C_5H_6N_2OS$	142.020113[d] (142.02008)
3a	284(16300) 353 (7500)	253 (9700) 277(11200) 376 (7800)	228 (9200) 275(13050) 329 (6300)	$C_4H_3F_2S_2$[f]	161.97216[d] (161.972214)
3o	285(16600) 349 (7450)	257(10000) 280(14600) 364 (8600)	274(14700) 323 (4300) 364 (4950)	$C_5H_6N_2S_2$	157.997165[d] (157.99724)
5	279(17000)	279(15700)	280(13000)	$C_4H_6N_2OS$	130.02019[d] (130.02008)
7	284(17300)	283(14000)	298(15200)	$C_5H_8N_2OS$	144.03558[d] (144.03574)
9	227(13300) 280(18900)	245(16800) 272(17200)	226(17900) 267(12100)	$C_4H_5N_3S$	127.02041[d] (127.020439)
11	256 (3800) 331(17200)	260 (3800) 334(13900)	215(16600) 261 (3600) 309(13800)	$C_5H_7N_3S$	141.03607[d] (141.036087)
13	255 (3400) 329(20300)	255 (3500) 329(20500)	315(18100)	$C_{10}H_{14}N_2O_5S$[f]	275.0731[e] (275.0702)
15	338(20800)	337(19800)	323(17000)	$C_{11}H_{16}N_2O_5S$	288.0781[d] (288.0780)
17	335(22100)	335(22100)	326(20000)	$C_9H_{14}N_2O_4S$	259.07530[e] (259.07525)
19	334(22400)	335(21000)	321(18900)	$C_{10}H_{13}FN_2O_3S$	260.06315[d] (260.06309)
21	339(18800)	327(17700)	324(18200)	$C_9H_{11}F_2N_2O_3S$	265.04552[e] (265.04585)

[a]0.01 N HCl

[b]0.05 M phosphate buffer

[c]0.01 N NaOH

[d]satisfactory EIMS exact mass was obtained for M^+

[e]satisfactory LSIMS exact mass was obtained for $M^+ + H$

[f]satisfactory elemental microanalyses were obtained for C ± 0.05, H ± 0.05, N ± 0.05.

its 4-thio analogue 21. These reduced yields for the dideoxynucleosides are due to their lower stability under the reaction conditions used.

Thiation of 2′,3′-dideoxy-3′-azidothymidine (AZT) (22), surprisingly, led to a complex series of reactions, the products of which included 4-thiothymine and 2′,3′-dideoxy-3′-amino-4-thiothymidine in minor quantities. Formation of the 3′-aminonucleoside is apparently due to the reductive action of the Lawesson reagent, which was recently shown to effectively reduce sulfoxides to the corresponding sulfides.[20b]

In conclusion, the Lawesson reagent in refluxing dioxane leads to thiation in high yields of the 2-keto group and 4-keto group of 2,4-diketopyrimidines, the 2-keto group of cytosine analogues and the 4-keto group of isocytosine and 1-substituted 2,4-diketopyrimidines, including nucleosides and 2′,3′-dideoxynucleosides, except azido nucleosides which undergo reduction of the azido group and cleavage of the glycosidic bond.

The facile thiation of dihydrouracils in THF at room temperature pointed to the possibility of similar thiation of labile 2′,3′-dideoxynucleosides at a lower temperature (b.p. of THF 65°C as co. to 100°C for dioxane). It was, in fact, found that the reaction of **18** and **20** with the Lawesson reagent in THF proceeded at 37°C, but high yields of the thiated nucleosides **19** and **21** were most conveniently obtained at reflux temperature. It is clear that THF is a better solvent for thiation of 5,6-dihydrouracils and the labile 5,6-dihydrouridines and pyrimidine 2′,3′-dideoxynucleosides, while 1,4-dioxane is adequate to the thiation of pyrimidinones and their ribo- and 2′-deoxyribonucleosides. The thiation of 2-keto group of pyrimidine nucleosides even with a large excess of Lawesson reagent in refluxing 1,4-dioxane or THF does not occur.

EXPERIMENTAL SECTION

Melting points (uncorr.) were determined on a Boetius microscope hot stage. TLC on Merck 60 F_{254} silica gel plates

(DC, 0.25 mm, No. 5715) and preparative (PSC, 2 mm, No. 5717) plates made use of the following solvents (v/v): (A) $CHCl_3$-MeOH, 9:1; (B) $CHCl_3$-MeOH, 85:15; (C) $CHCl_3$-MeOH, 7:3; (D) $CHCl_3$-MeOH, 1:1. 1,4-Dioxane and tetrahydrofuran were dried by passage through a column of Merck alkaline Al_2O_3 (Brockman activity I) and stored over 4 Å molecular sieves. The Lawesson reagent was a product of Aldrich (The reagent is extremely sensitive to the moisture and fresh samples must be used in the thiation procedures). High-resolution mass spectra - HRMS (EI) for pyrimidinones was carried out on a Finigan MAT spectrometer, and liquid matrix secondary ion mass spectra - HRMS (LSIMS) for nucleosides with an AMD-604 spectrometer. UV spectra were recorded on a Cary model 3. High resolution ^{1}H-NMR spectra were recorded on Bruker 500 MHz in D_2O and DSS as internal standard. Chemical shifts (δ) are reported in ppm, J values are given in Hz. All evaporations were under vacuum at a temp. < 35°C.

5-Fluoro-4-thiouracil (2a)

To a solution of 5-fluorouracil (**1a**) (1.30 g, 10 mmol) in 50 ml anhydrous 1,4-dioxane was added the Lawesson reagent (2.25 g, 5.57 mmol). The mixture was heated under reflux for 2 hours with exclusion of moisture, and progress of the reaction monitored by TLC with solvent A. Solvent was removed under vacuum to give a residual oil, which was dissolved in boiling water, insoluble material removed by filtration, and the clear filtrate stored for crystallization. The product was recrystallized from ethanol to give 1.32 g of **2a**. Yield 89%; m.p. 276-278°C (lit.[11,12] 277-278°C); TLC: R_f 0.36 in solvent A.

6-Methyl-4-thiouracil (2b)

Compound **2b** was prepared from **1b** as described above for **2a** and isolated as follows: the reaction mixture was cooled to promote crystallization, the crystals were collected by filtration, and washed with cold dioxane. An analytical sample was obtained by recrystallization from water.; Yield 84%; m.p. 338-340°C (lit.[21] 339-341°C); TLC: R_f 0.48 in solvent A.

6-Ethyl-4-thiouracil (2c)

2c was prepared from 1c as described for 2a and isolated as described for 2b. Yield 85%; m.p. 199-202°C dec. (lit.[22] 223°C dec.); TLC: R_f 0.57 in solvent A.

6-Propyl-4-thiouracil (2d)

2d was prepared from 1d as described for 2a and isolated as described for 2b. Yield 86%; m.p. 186-187°C (lit.[22] 193°C); TLC: R_f 0.67 in solvent A.

6-Isopropyl-4-thiouracil (2e)

2e was prepared from 1e as described for 2a and isolated as described for 2b. Yield 86%; m.p. 172-174°C; TLC: R_f 0.72 in solvent A; Anal. Calc. for $C_7H_{10}N_2OS$ (170.2): C, 49.38; H, 5.92; N, 16.45. Found: C, 49.92; H, 6.28; N, 16.32.

5-Fluoro-6-methyl-4-thiouracil (2f)

2f was prepared from 1f and isolated as described for 2a. Yield 80%; m.p. 360°C dec.; TLC: R_f 0.43 in solvent A; Anal. Calc. for $C_5H_5FN_2OS \times H_2O$ (178.2) C, 33.69; H, 3.95; N, 15.72. Found: C, 33.88; H, 3.67; N, 16.32.

5,6-Dimethyl-4-thiouracil (2g)

2g was prepared from 1g as described for 2a and isolated as described for 2b. Yield 81%; m.p. 273°C dec.; TLC: R_f 0.62 in solvent A; Anal. Calc. for $C_6H_8N_2OS \times H_2O$ (174.2): C, 41.36; H, 5.78; N, 16.08. Found: C, 41.94; H, 5.57; N, 16.17.

5-Chloro-6-methyl-4-thiouracil (2h)

2h was prepared from 1h and isolated as described for 2a. Yield 88%; m.p. 263°C dec.; TLC: R_f 0.47 in solvent A; Anal. Calc. for $C_5H_5ClN_2OS$ (176.6): C, 33.99; H, 2.85; N, 15.86. Found: C, 33.55; H, 3.01; N, 16.04.

4-Thio-6-trifluoromethyluracil (2i)

2i was prepared from 1i and isolated as described for 2a. Yield 85%; m.p. 165-166°C; TLC: R_f 0.10 in solvent A; Anal. Calc. for $C_5H_3F_3N_2OS$ (196.2): C, 30.61; H, 1.54; N, 14.28. Found: C, 30.82; H, 1.55; N, 14.06.

6-Chloromethyl-4-thiouracil (2j)

2j was prepared from 1j and isolated as described for 2a. Yield 90%; m.p. 197°C; TLC: R_f 0.50 in solvent A; Anal. Calc. for $C_5H_5ClN_2OS$ (176.6): C, 33.99; H, 2.85; N, 15.86. Found: C, 33.62; H, 2.70; N, 15.41.

5-Fluoro-6-fluoromethyl-4-thiouracil (2k)

2k was prepared from 1k as described for 2a and isolated as described for 2b. Yield 82%; m.p. 157 dec.; TLC: R_f 0.42 in solvent A. Anal. Calc. for $C_5H_4F_2N_2OS$ (178.2): C, 33.70; H, 2.26; N, 15.73. Found: C, 33.44; H, 2.21; N, 15.51.

1,3-Dimethyl-4-thiouracil (2l)

2l was prepared from 1l as described for 2a and isolated as follows: the reaction mixture was concentrated under vacuum, and the product isolated on preparative silica gel plates (Merck PSC Kieselgel 60 F_{254}) with solvent A, eluted with solvent D, the eluate brought to dryness, and the residue crystallized from EtOH. Yield 78%; m.p. 130-131°C (lit.[23] 132-133°C); TLC: R_f 0.92 in solvent A. **1,3-Dimethyl-5-fluoro-4-thiouracil (2m)**

2m was prepared from 1m as described for 2a and isolated as described for 2l. Yield 81%; m.p. 168°C (lit.[24] 171-172°C); TLC: R_f 0.91 in solvent A.

1,3-Dimethyl-4-thiothymine (2n)

2n was prepared from 1n as described for 2a and isolated as described for 2l. Yield 79%; m.p. 177-179°C.; TLC: R_f 0.94 in solvent A.

4-Thiothymine (2o)

2o was prepared from 1o as described for 2a and isolated as described for 2b. Yield 70%; m.p. 305-306°C (lit.[25] 306-307°C); TLC: R_f 0.49 in solvent A.

2,4-Dithio-6-methyluracil (2p)

2p was prepared from 1p as described for 2a and isolated as described for 2b. Yield 90%; m.p. 352°C (lit.[12] 354-356°C); TLC: R_f 0.85 in solvent A.

2,4-Dithio-5-fluorouracil (3a)

Method A:

To the solution of 1a (1.30 g, 10 mmol) in 50 ml anhydrous 1,4-dioxane was added the Lawesson reagent (4.50 g, 11.14 mmol). The mixture was heated under reflux for 16 hours with exclusion of moisture, and progress of the reaction monitored by TLC with solvent A. The cooled reaction mixture was brought to an oil under vacuum, taken up in boiling 2-butanol, filtered, and again cooled to promote crystallization. An analytical sample was obtained by recrystallization from ethanol to give 1.46 g of 3a. Yield 90%; m.p. 227-229°C (lit.[26] >300°C); TLC: R_f 0.40 in solvent A; Anal. Calc. for $C_4H_3FN_2S_2$ (161.9): C, 29.63; H, 1.86; N, 17.29. Found: C, 30.07; H, 2.09; N, 16.83.

Method B:

3a was prepared from 2a as described for 3a (Method A). Yield 89%.

2,4-Dithiothymine (3o)

3o was prepared from 1o as described for 3a (method A) and isolated as described for 21. Yield 73%; m.p. 260-263°C (lit.[27] 284-285°C); TLC: R_f 0.68 in solvent A.

5,6-Dihydro-4-thiouracil (5)

A solution of 5,6-dihydrouracil (4) (114 mg, 1 mmol) and Lawesson reagent (808 mg, 2 mmol) in 30 ml anhydrous tetrahydrofuran was stirred at room temp. for 16 h, following which TLC (solvent A) demonstrated disappearance of 4. The clear solution was reduced to 4 ml and cooled, leading to a precipitate which was collected by filtration, washed with THF and dried over P_2O_5 to yield 68 mg. The filtrate was deposited on two 40x20 cm silica gel plates. Development with solvent A gave a pale yellow band with R_f 0.55, which was eluted with solvent D and brought to dryness over P_2O_5 to give 27 mg of 5 (overall yield 73%). Recrystallization from methanol yielded an analytical sample, m.p. 191-192°C (Lit.[19] 191-193°C). TLC: R_f 0.60 in solvent A. UV (EtOH): λ_{max} 279 (ϵ 16800). Lit.[19] λ_{max} 279.5 (ϵ 16900).

5,6-dihydro-1-methyl-4-thiouracil (7)

The foregoing procedure, applied to 5,6-dihydro-1-methyluracil (6) led to 7 (113 mg, 83%), m.p. 194-195°C (Lit.[19] 193-195°C). TLC: R_f 0.84 in solvent A. UV (EtOH): λ_{max} 282 (ε 15900). Lit.[19] λ_{max} 281 (ε 16200).

2-Thiocytosine (9)

9 was prepared from cytosine (8) as described for 3a (Method A) and isolated as described for 21. Yield 80%; m.p. 263-265°C (lit.[28] 278°C dec.); TLC: R_f 0.11 in solvent A.

6-Methyl-4-thioisocytosine (11)

11 was prepared from 6-methylisocytosine (10) as described for 2a and isolated as follows: Solvent was removed under vacuum to a residual oil, which was dissolved in dilute HCl, insoluble material removed by filtration and product was precipitated with 5% aqueous ammonia. Yield 73% m.p.>190°C dec. (lit.[29] 321°C); TLC: R_f 0.51 in solvent A.

"One-pot" thiation of nucleosides. General procedure

6-Methyl-4-thiouridine (13)

To 6-methyluridine (12) (0.26 g, 1 mmol) in 4 ml anhydrous pyridine was added 2 ml freshly distilled acetic anhydride and the mixture left overnight at room temperature. Solvent was then removed and the residue dried azeotropically with anhydrous toluene (3 x 5 ml). The residue was dissolved in 5 ml anhydrous 1,4-dioxane, and 1.2 equivalents (0.24 g) of Lawesson reagent was added. The mixture was heated under reflux for 2 h, at which point TLC (solvent B) demonstrated disappearance of 12. The mixture was cooled to room temperature, followed by addition of 5 ml conc. NH_4OH, and left overnight at room temperature, leading to the deblocked nucleoside 13 (TLC, solvent A). The reaction mixture was concentrated to small volume, deposited on 4 preparative silica gel plates, and developed with solvent A. The spot with R_f 0.25 was eluted with solvent D, the eluate brought to dryness and the residue dried 3 x from anhydrous toluene, then over P_2O_5 under vacuum, to give 0.23 g (82%) m.p. 161-163°C; TLC: R_f 0.27 in

solvent A. ^{1}H-NMR: δ 2.37(s, 3H, CH_3), 3.75(dd, 1H, H-5"), 3.88(dd, 1H, H-5',$J_{5',5"}$=12.38,), 3.98(m, 1H, H-4',$J_{4',5'}$=3.01, $J_{4',5"}$=6.27,), 4.39(dd, 1H, H-3', $J_{2',3'}$=6.44, $J_{3',4'}$=7.15,), 5.69(d, 1H, H-1' $J_{1',2'}$=3.41,), 6.49(d, 1H, H-5); Anal. Calc. for $C_{10}H_{14}N_2O_5S$ x ½ EtOH (297.3): C, 44.44; H, 5.76; N, 9.42. Found: C, 44.01; H, 5.77; N, 9.98.

1-(β-D-ribofuranosyl)-5-ethyl-4-thiouracil (15)

This was prepared from 5-ethyluridine (14), as for 13, above, as a glass, in 76% yield; TLC: R_f 0.25 with solvent A, R_f 0.44 with solvent B. ^{1}H-NMR : δ 1.2(t, 3H, CH_3-5, J=14.46), 2.62(q, 2H, CH_2-5, J=22.51), 3.9(dd, 1H, H-5", $J_{5',5"}$=-12.78), 4.03(dd, 1H, H-5'), 4.22(m, 1H, H-4', $J_{4',5'}$=2.84, $J_{4',5"}$=3.66), 4.32(dd, 1H, H-3', $J_{3',4'}$=6.11), 4.42(dd, 1H, H-2',$J_{2',3'}$=5.24), 5.97(d, 1H, H-1',$J_{1',2'}$=3.59), 7.86(s, 1H, H-6).

1-(2-Deoxy-β-D-ribofuranosyl)-5-methyl-4-thiouracil (17)

This was prepared from thymidine (16), as for 13, yield 68%, as a yellow amorphous powder, m.p. 123-125°C (lit.[30] 125-127°C), TLC: R_f 0.36 in solvent A. ^{1}H-NMR: δ 2.08(br s, 3H, CH_3-5), 2.39(m, 1H, H-2',$J_{2',2"}$=-14.14), 2.45(m, 1H, H-2"), 3.78(as q, 1H, H-5",$J_{5',5"}$=12.51) 3.87(as q, 1H, H-5'), 4.06(m, 1H, H-4',$J_{4',5'}$=3.58, $J_{4',5"}$=4.86) 4.47(m, 1H, H-3',$J_{2',3'}$=6.79, $J_{2",3'}$=4.38, $J_{3',4'}$=4.12), 6.24(t, 1H, H-1',$J_{1',2'}$=6.57, $J_{1',2"}$=6.55), 7.76(br s, 1H, H-6).

1-(2,3-dideoxy-3-fluoro-β-D-ribofuranosyl)-5-methyl-4-thiouracil (19)

19 was prepared from 2'3'-dideoxy-3'-fluoro-5-methyluridine (18), as for 13 in 60% yield. In another experiment the thiation of 18 in refluxing THF gave 73% yield of 19; TLC: R_f 0.74 in solvent A. An analytical sample, crystallized from EtOH-toluene, gave m.p. 131-132°C. ^{1}H-NMR: δ 2.10(d, 3H, CH_3-5, J=1.89), 2.41(d o, 1H, H-2', $J_{1',2'}$=9.0, $J_{2',2"}$=-14.94, $J_{2',3"}$=0.6), 2.72(d o, 1H, H-2"), 3.83(d, 1H, H-5',$J_{4',5'}$=4.38), 3.83(d, 1H, H-5",$J_{4',5"}$=4.38), 4.43(d t, 1H, H-4',$J_{3',4'}$=0.6), 5.36(d d, 1H,H-3',$J_{2',3'}$=5.48), 6.32(d d, 1H, H-1', $J_{1',2'}$=9.0), 7.76(d, 1H, H-6, J=0.87).

1-(2,3-Dideoxy-3-fluoro-β-D-ribofuranosyl)-5-fluoro-4-thiouracil (21)

21 was prepared from 2'3'-dideoxy-3',5-difluorouridine (20), as for 13 in 62% yield as a glass. In another experiment the thiation of 20 in refluxing THF gave 75% yield of 21; TLC: R_f 0.63 in solvent A. ^{1}H-NMR δ 2.29(m, 1H, H-2', $J_{1',2'}$=8.86, $J_{2',3'-F}$=39.40), 2.60(d m, 1H, H-2", $J_{2',2"}$=-14.92, $J_{2",3'-F}$=20.80), 3.79(d, 2H, H-5', H-5"), 4.30(d m, 1H, H-4', $J_{3',4'}$=0.50, $J_{4',5'+4',5"}$=6.00, $J_{4',3'-F}$=37.50), 5.27(d m, 1H, H-3', $J_{2',3'}$=4.66, $J_{2",3'}$=0.80, $J_{3',3'-F}$=48.36), 6.27(tr d, 1H, H-1', $J_{1',2"}$=5.52, $J_{1',5F}$=1.60), 8.13(d, 1H, H-6, $J_{6,5F}$=4.72).

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