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Synthesis of *C*-5 and *N*-3 Arenesulfenyl Uridines. Preparation and Properties of a New Class of Uracil Protecting Group

C.J. WELCH, H. BAZIN, J. HEIKKILÄ and J. CHATTOPADHYAYA*

Bioorganic Chemistry Laboratory, Department of Microbiology, Box 581, Biomedical Center, Uppsala University, S-751 23 Uppsala, Sweden

The nature and position of the ring substituent of an arenesulfenyl chloride control the regiospecific formation of either a *C*-5 substituted product, as in compounds *3a–3d*, or a *N*-3 substituted product, as in compounds *2a–2c*. Arenesulfenyl groups have been subsequently found to successfully protect the urethane function of the uracil residue as exemplified by the synthesis of 2′-*O*-methyl uridine and oligoribonucleotide building blocks.

The reaction of an electrophile across the C-5–C-6 double bond of uridine is favoured at *C*-5 position because of the enamine-like contribution from the lone-pair of electrons of *N*-1, as depicted in Scheme 1.

Several such electrophilic substitution reactions are reported in the literature.[1–8] We herein report our studies on the reaction of arenesulfenyl chlorides with 2′,3′,5′-tri-*O*-acetyluridine *1* in dry pyridine solution at room temperature. We subsequently show some applications of these reactions in the chemical synthesis of 2′-*O*-methyl uridine and in the preparation of building blocks for the chemical synthesis of oligoribonucleotides.

In the first part of this study it has been shown that the nature and position of the ring substituent of the arenesulfenyl chloride entirely controls the regiospecificity of the formation of either *N*-3-arenesulfenylated products, as in *2a–2c*, or *C*-5 substituted products as shown in *3a–3d*. Thus (*2a*) to (*2c*) were the sole products formed, isolated in 87, 90 and 80 % yields, respectively, upon treatments of *1* with an excess (2 equivalents) of 2-nitrobenzenesulfenyl (Nbs) chloride, 2-nitro-4-toluenesulfenyl (Nts) chloride and 2,4-dinitrobenzenesulfenyl (Dnbs) chloride, respectively. In contrast, the treatment of tri-*O*-acetyl uridine *1* with benzene-, 4-toluene-, 4-chlorobenzene- and 4-nitrobenzenesulfenyl chlorides, gave under similar reaction conditions, the corresponding *C*-5 substituted products, *3a–3d* in 66, 80, 74, 64 % yields, respectively, in conjuction with the formation of a varied amount of 5-chloro-2′,3′,3′-tri-*O*-acetyluridine *4* (5–10 %). The yields of *4* varied with the quality of arenesulfenyl chlorides (GLC). It is interesting to note that no *N*-3 substituted product was formed in the latter reactions as judged by TLC and NMR studies. These studies clearly show that it is necessary to have an *ortho* nitro substituent in the arenesulfenyl chlorides, as in Nbs–Cl, Dnbs–Cl and Nts–Cl, in order to form *N*-3

* To whom correspondence should be addressed.

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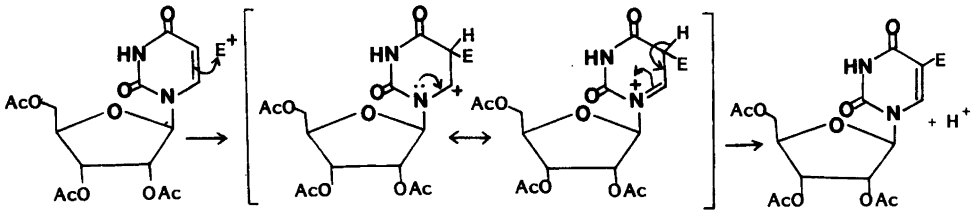
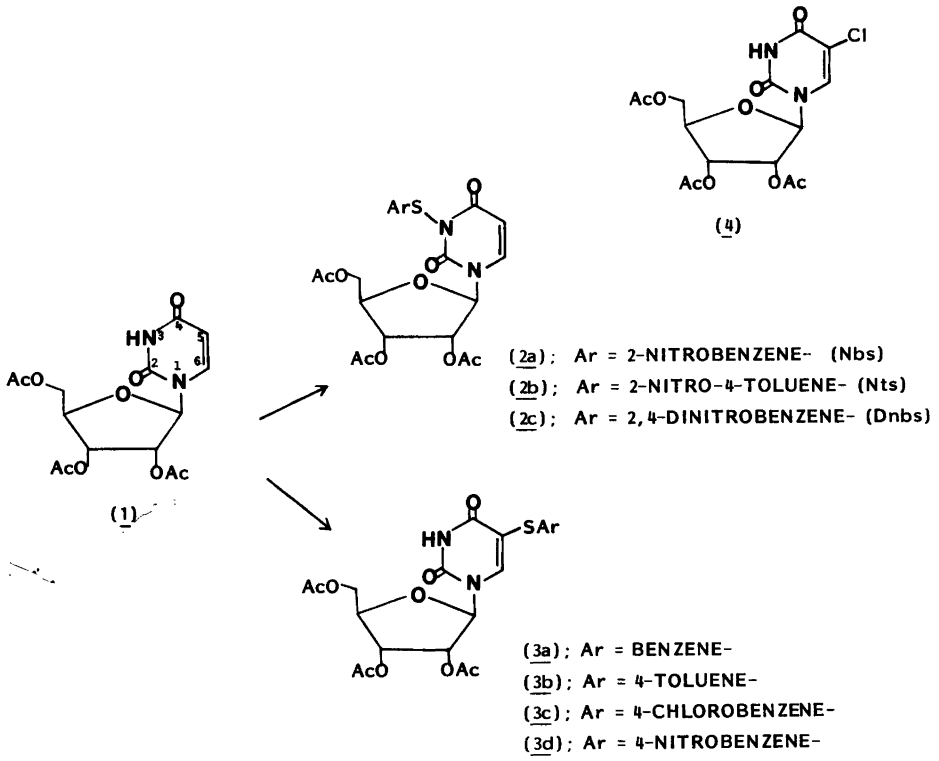


SCHEME 1



substituted products as shown in *2a–2c*, respectively. No 5-chloro- or 5-arene substituted products were formed in these reactions as judged by TLC and NMR. It is difficult to rationalise such an exclusive formation of *ortho*-nitrobenzenesulfenyl substituted product at *N*-3, as opposed to the formation of only *C*-5 *para*-nitrobenzenesulfenylated product *3d* with 4-nitrobenzenesulfenyl chloride, without involving an extra stabilisation factor such as an internal charge transfer complex. A comparison of the pK_a[9] of 4-nitrophenol and 2-nitrophenol, 7.15 and 7.23, respectively, clearly rules out any significant difference in mesomeric contribution between *ortho* nitro and *para* nitro substituents through the benzene ring which might affect the electrophilicities of sulphur preferentially either in 4-nitro or 2-nitrobenzenesulfenyl chlorides. It, therefore, appears likely that the lowering of the activation energy, by an intramolecular force, would be expected to be the major factor in the observed electrophilic reaction of uridine at *N*-3.

Having explored these chemoselective reactions, we then diverted our attention to employ the *N*-3 substituted derivatives, as uracil protecting groups in the chemical synthesis of naturally occurring nucleosides and nucleotides.

Regioselective chemical transformation of a hydroxyl function of the ribonucleoside moiety of uridine warrants protection of urethane function of the uracil residue. This has been shown to be clearly desirable, especially for the preparation of building blocks in the chemical synthesis of oligoribonucleotides[10] or in the preparation of 2′-*O*-methyluridine[11] which occurs as a minor tRNA component.

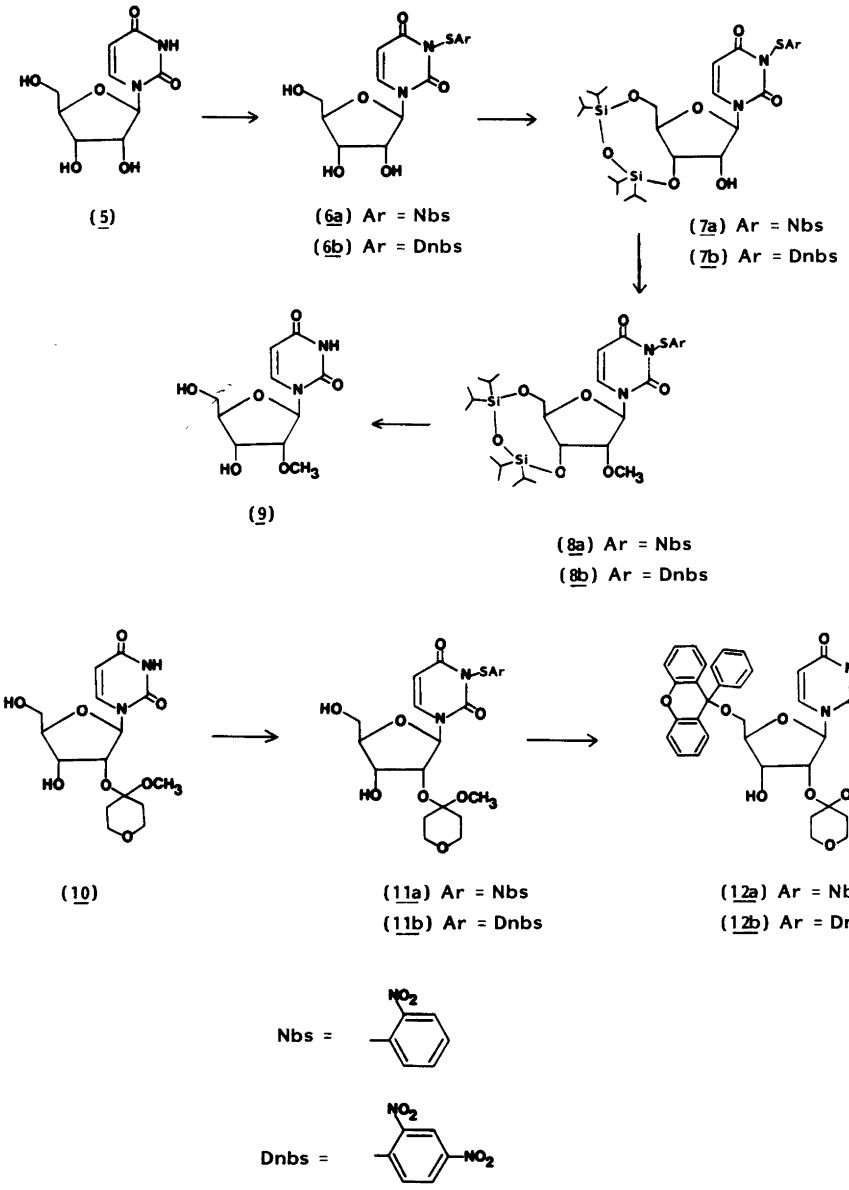
The groups that have been prescribed so far for the protection of the uracil moiety include: 4-*O*-aryl-,[11] 3-*N*-2,2,2-trichloro-*t*-butyloxycarbonyl-,[12] acyl-,[13,14] methoxyethoxymethyl-[15] and 2-(4-nitrophenyl)ethyl-[16] groups. In the present work, we propose an alternative set of *N*-3-arenesulfenyl protective groups for this purpose which may be prepared in high yields by a "one-pot" synthesis. Letsinger and his co-workers first demonstrated[17] that the reaction of an excess of 2,4-dinitrobenzenesulfenyl chloride with thymidine in pyridine furnished tris-2,4-dinitrobenzenesulfenyl derivative; the extra arenesulfenyl group was introduced into the thymine ring, and could be conveniently removed by treatment with thiophenol in pyridine at room temperature. However, these workers neither targeted this observation to a synthetic problem, presumably due to problems of preparation of a *N*-3 protected uridine block specifically with free hydroxyl functions, nor have they ellucidated the actual location of the 2,4-dinitrobenzenesulfenyl group in the thymine skeleton.

In our procedure, we have developed a "one-pot" synthesis of such *N*-3-(2-nitrobenzene)sulfenyl-(Nbs)-(*6a*) and 2,4-dinitrobenzenesulfenyl- (Dnbs) (*6b*) uridine derivatives involving trimethylsilylation[18] of uridine in dry pyridine followed by the addition of 2-nitro- or 2,4-dinitrobenzenesulfenyl chloride *in situ* at room temperature and then methanolysis to give pure *6a* and *6b* in 88 and 93 % yields, respectively. To our knowledge, the present preparation of the above *N*-3-arenesulfenyluridines, *6a* and *6b*, with free hydroxyl groups constitutes their first report in the literature.

The unambiguous evidence for the *N*-3 postition of the arenesulfenyl group in the uracil moiety was obtained by ^{13}C–NMR studies. The chemical shifts for the sp^2 hybridized C-4 and C-2 carbons in uridine *5* are assigned at 163.06 and 150.65 ppm, respectively, downfield from TMS. These two sp^2 hybridized carbons resonate at 161.95 for C-4 and at 151.33 ppm for C-2 in the compound *6a*; similarly in compound *2b*, the C-4 and C-2 absorb at 160.32 and 149.58 ppm, respectively. These observations clearly substantiate our structural assignment that the original hybridization states of C-4 & C-2 of uridine have been retained in these new arenesulfenylated derivatives as shown in (*6a*) and (*6b*).

The Nbs and Dnbs groups, in *6a* and *6b*, respectively, are stable in dry pyridine solution containing triethylamine (10 equiv.); however, the half-lives for their removal in 10 and 50 % aqueous pyridine solution are 40 and 100 min and 8 and 36 min respectively. The Dnbs and Nbs groups are stable in acidic conditions such as 2 % 4-toluenesulfonic acid monohydrate in 30 % methanol–chloroform mixture or in 80 % acetic acid at room temperature. Both Nbs and Dnbs groups in *6a* and *6b* were found to be relatively unstable, $t_{1/2}$ 25 and 1 min, respectively, in the presence of tetrabutylammonium fluoride (0.2 M, 2 equiv. in dry tetrahydrofuran at room temperature).

The compounds *6a* and *6b* have been subsequently employed for the synthesis of 2′-*O*-methyluridine *9*, in 60 and 62 % overall yields in four steps, which was earlier prepared by a Japanese group of workers[12] in seven steps in 29 % overall yield.



The intermediates, *7a* and *7b*, for the synthesis of the above target compound were prepared in 83 and 77 % yields, respectively, by the treatment of *6a* or *6b* with a slight excess of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPDSiCl_2$)[24] in dry pyridine solution following a procedure reported in the literature.[26] These partially protected intermediates, *7a* and *7b*, were then methylated with iodomethane (10 equiv.) in dry acetone (10 ml/mmol) in the presence of silver oxide (8 equiv.) at room temperature for 16 h. A standard work up followed by chromatography on a short column of silica gel gave *8a* and *8b* in 94 and 86 % yields, respectively. The TIPDSi and arenesulfenyl groups were then removed simul-

taneously from (*8a*) and (*8b*) with an excess of n-tetrabutylammonium fluoride in dry tetrahydrofuran ([F-]=0.1M; 2 equiv.) for 5 min, followed by a treatment with triethylammonium thiocresolate (0.5 M; 3 equiv.) for 1 min, giving a quantitative conversion (TLC) to 2′-*O*-methyluridine *9* which was isolated in 92 and 83 % yields, respectively. Spectroscopic data substantiated the structure of all intermediates and the target compound.

The above sequences of chemical reactions finally convinced us that either of these arenesulfenyl groups at *N*-3 position would fulfil the requirements of an *N*-3 protecting group of the uracil moiety in uridine for tRNA and mRNA synthesis, provided one could avoid a synthetic sequence that involves use of fluoride ions as a reagent. We have thus prepared both 3-*N*-Nps-2′-*O*-(4-methoxytetrahydropyranyl)-(*11a*) and 3-*N*-Dnbs-2′-*O*-(4-methoxytetrahydropyranyl)uridine (*11b*) employing a "one-pot" synthesis identical to the procedure used for the synthesis of *6a* and *6b*, starting from 2′-*O*-(4-methoxytetrahydropyranyl)uridine (*10*)[26]. Following the sequence of such a "one-pot" procedure and a column chromatographic purification step, *11a* and *11b* were obtained in 65 and 63 % isolated yields. Subsequently, the 5′-hydroxyl functions of *11a* and *11b* were selectively protected with the 9-phenyl-9H-xanthen-9-yl (pixyl) group[25] using 9-chloro-9-phenylxanthene (1.1 equiv.) in dry pyridine solution for 40 min at room temperature. Thus partially protected blocks *12a* and *12b* were obtained in 88 and 89 % yields, respectively, after a standard work up and purification step. Preparation of such appropriately protected nucleoside building blocks, constitutes an important step for the selective introduction of a phosphate ester function for oligoribonucleotide synthesis.[26] Further work is now in progress in this laboratory for a total chemical synthesis of $tRNA^{Phe}$ using such uridine building blocks.

EXPERIMENTAL

2-nitrobenzenesulfenyl chloride and 2-nitro-4-toluenesulfenyl chloride were prepared according to a standard procedure.[19]

2,4-dinitrobenzenesulfenylchloride was purchased from Jansen Chemica (Belgium) and recrystallised from dry carbontetrachloride before use. Benzene-, 4-toluene- and 4-chlorobenzenesulfenyl chlorides were all prepared both by direct chlorination of the corresponding mercaptan with sulfuryl chloride[20] and by reaction of the disulfide with elemental chlorine.[21]

4-nitrobenzenesulfenylchloride was only prepared by the former method. 2′,3′,5′-tri-*O*-acetyluridine was prepared by a reaction of a dry pyridine solution of uridine and acetic anhydride.

Reactions were monitored by TLC using Merck pre-coated silica gel 60 F_{254} plates eluting with 10 % ethanol-chloroform mixture. Column chromatographic purifications were carried out using Kiesegel 60 G.[22] ^1H NMR spectra were recorded using a Hitachi-Perkin Elmer R600 spectrophotometer. ^{13}C NMR were recorded using a Jeol FX 90 Q spectrometer, observing at 23.7 kHz. NMR spectra were recorded using deuterochloroform as solvent, unless otherwise stated, and tetramethyl silane as internal standard. Ultraviolet spectra were recorded with a Cary 2200 spectrometer.

N-3-(2-nitrobenzene)sulfenyl-2′,3′,5′-tri-O-acetyluridine(2a): Compound *1* (1 g, 2.7 mmol) was coevaporated to dryness with pyridine, then dissolved in dry pyridine (25 ml). To the solution was added 0.67 g (3.5 mmol) 2-nitrobenzenesulfenyl chloride, the solution was stirred for 20 min by which time TLC showed complete consumption of compound *1* and formation of a product with a higher R_f. The reaction mixture was then worked up by pouring the solution into saturated sodium bicarbonate solution (150 ml) and extracting with chloroform (3×50 ml). The combined organic layers were evaporated to dryness, coevaporated with toluene several times to remove all traces of pyridine. Then the product was purified by column chromatography, eluting with a 50 % dichloromethane-chloroform

mixture. The product was precipitated from light petroleum and dried *in vacuo*. Yield: 1.23 g (87 %). R_f=0.62; ^{1}H NMR: δ 8.36 (*dd*, 9.0 & 1.2, 1 H) H-3 of 2-nitrobenzene; 7.57 (*d*, 8.2, 1 H) H-6; 7.63–6.93 (*m*, 3 H) H-4,-5 and -6 of Nbs group; 6.01 (*d*, 8.2, 1 H) H-5; 5.98 (*d*, 4.8, 1 H) H-1′; 5.40 (*d*, 4.8, 1 H) H-2′; 5.34 (*m*, 1 H) H-3′; 4.38 (*bs*,3 H) H-4′ and 5′; 2.11 (*s*, 6 H) and 2.03 (*s*, 3 H) acetyl groups. ^{13}C NMR δ 170.1, 169,9 169.6, 161.9, 151.5, 143.0, 139.0, 134.9, 125.9, 123.2, 102.4, 89.6, 80.3, 73.1, 70.3, 63.0, 20.8, 20.4.

UV (ethanol): pH 7 λ_{max} 364 nm (ε=4400), 266 nm (ε=13 700); 238 nm (ε=19 400); pH 2 λ_{max} 365 nm (ε=4300), 265 nm (ε=13600), 238 nm (ε=17 800); pH 13 λ_{max} 396 nm (ε=3100), 236 nm (ε=17 100).

N-*3-(2-nitro-4-toluene)sulfenyl-2′,3′,5′-tri-O-acetyluridine (2b)*. *1* (0.37 g, 1 mmol) and 2-nitro-4-toluenesulfenyl chloride (0.29 g, 1.4 mmol) were reacted together using the same condition as for compound *2a*. After 3 h the reaction had progressed approximately 90 %, as estimated by TLC, use of a larger excess of the reagent, or a longer reaction time did not appear to give any further reaction. Work up and purification of the product was as for *2a*. Yield: 0.43 g (80 %). R_f 0.56.

^{1}H NMR δ 8.14 (*bs*, 1 H) H-2 of arene system; 7.52 (*d*, 8.2, 1 H) H-6; 7.46–7.28 (*m*, 1 H) H-5 of arene system; 6.84 (*d*, 8.2, 1 H) H-6 of arene system; 5.97 (*d*, 8.2, 1 H) H-5; 5.97 (*d*, 3.8, 1 H) H-1′; 5.34 (*m*, 2 H) H-2′ and 3′; 4.36 (*bs*, 3 H) H-4′ and 5′; 2.40 (*s*, 3 H) aromatic methyl group; 2.11 (*s*, 6 H) and 2.03 (*s*, 3H) acetyl groups.

^{13}C NMR δ 170.0, 169.6, 161.9, 151.4, 138.9, 136.6, 136.0, 135.1, 125.8, 122.9, 102.3, 89.7, 80.2, 73.0, 63.0, 20.8, 20.4.

UV (ethanol): pH λ_{max} 376 nm (ε=4500), 264 nm (sh) (ε=13 000), 235 nm (ε=18 000). pH 7 λ_{max} 374 nm (ε=4600), 264 nm (sh) (ε=13 600), 235 nm (ε=18 700). pH 13 λ_{max} 408 nm (ε=3400), 255 nm (sh) (ε=15 400), 237 nm (ε=17 800).

N-*3-(2,4-dinitrobenzene)sulfenyl-2′,3′,5′-tri-O-acetyluridine (2c)*. *1* (0.37 g, 1 mmol) and 2,4-dinitrobenzenesulfenyl chloride (0.47 g, 2 mmol) were reacted together using the same conditions as for *2a*. The reaction appeared complete after 1 h. Work up and purification identical to that in *2a* yielded the desired compound, 0.51 g (90 %); R_f 0.65.

^{1}H NMR δ 9.19 (*d*, 2.8, 1 H) H-3 of Dnbs group; 8.45 (*dd*, 9.6 and 2.8, 1 H) H-5 of Dnbs; 7.60 (*d*, 8.1, 1 H) H-6; 7.21 (*d*, 9.6, 1 H) H-6 of Dnbs; 6.06 (*d*, 8.1, 1 H) H-5; 5.99 (*m*, 1 H) H-1′; 5.36 (*m*, 2 H) H-2′ and −3′; 4.38 (*bs*, 3 H) H-4′ and −5′; 2.11 (*s*, 6 H) and 2.03 (*s*, 3 H) acetyl groups.

^{13}C NMR: δ 170.1, 169.7, 161.5, 151.2, 146.4, 145.5, 142.7, 139.3, 128.2, 124.9, 121.4, 102.3, 89.6, 80.5, 73.3, 70.4, 63.1, 20.8, 20.4.

UV (ethanol): pH2 λ_{max} 350 nm (sh) (ε=5900), 310 nm (ε=10 100), 264 nm (ε=17 400); pH 7 λ_{max} 348 nm (sh) (ε=6000), 309 nm (ε=10 600), 264 nm (ε=17 600); pH 13 λ_{max} 331 nm (sh) (ε=8100), 258 nm (ε=16 100).

5-benzenesulfenyl-2′,3′,5′-tri-O-acetyluridine (3a). 2′,3′,5′-Triacetyl uridine (*1*) (1 g, 2.7 mmol) was coevaporated with pyridine *in vacuo*, then dissolved pyridine (25 ml). Benzenesulfenyl chloride was added to the reaction vessel and the solution was stirred for 36 h at 20 °C, by which time complete consumption of *1* was observed (TLC). The reaction mixture was worked up in a similar manner to that of *2a*, and purified by silica gel chromatography. The two products were eluted with a mixture of dichloromethane and chloroform (1:1; v/v). The product which eluted first was characterised as the title compound. Yield: 0.85g; R_f 0.61.

^{1}H NMR: δ 7.96 (*s*, 1 H) H-6; 7.28 (*bs*, 5 H) phenyl group; 6.08 (*d*, 4.8, 1 H) H-1′; 5.36 (m, 2 H) H-2′ and −3′; 4.36 (*bs*, 3 H) H-4′ and −5′; 2.13 (*s*, 9 H) acetyl groups.

^{13}C NMR: δ 170.1, 169.6, 160.8, 150.1, 144.4, 133.2, 132.8, 129.8, 129.2, 107.9, 87.5, 80.2, 73.1, 70.2, 63.0, 20.7, 20.5, 20.4.

UV (ethanol): pH 2 λ_{max} 300 nm (sh) (ε=4100); 251 nm (ε=13 900). pH 7 λ_{max} 300 nm (sh) (ε=4000); 252 nm (ε=14 800). pH 13 λ_{max} 276 nm (sh) (ε=8500); 253 nm (ε=13 500).

Mass spectroscopy: $M^{\cdot+}$+1 at *m/z* 479 (17.9 %). The product which eluted last was identified as 5-chloro-2′-3′-5′-tri-*O*-acetyluridine *4* and was identical to the compound prepared by reaction of 2′,3′,5′-tri-*O*-acetyluridine with molecular chlorine in acetic acid.[23] (Yield: 0.09 g; 8 %).

5-(4-toluene)sulfenyl-2′,3′,5′-tri-O-acetyluridine (3b). *1* (0.48 g, 1.3 mmol) and 4-toluenesulfenyl chloride (0.54, 4 mmol) were reacted using conditions identical to that of the preparation of *3a*. After 48 h, the reaction appeared to be complete (TLC). The mixture was

worked up and purified in a similar fashion to that of *2a*. Yield 0.58 g (90 %). R_f 0.48; ^{1}H NMR δ 7.83 (*s*, 1 H) H-5; 7.28–7.16 (*m*, 4 H) aromatic protons; 6.10 (*d*, 4.8, 1 H) H-1′; 5.34 (*m*, 3 H) H-2′ and −3′; 4.32 (*bs*, 3 H) H-4′ and −5′; 2.31 (*s*, 3 H) aromatic methyl; 2.13 (*s*, 9 H) acetyl groups.

^{13}C NMR δ 170.2, 169.7, 160.9, 150.3, 142.8, 137.2, 130.0, 129.7, 109.8, 87.3, 80.2, 73.1, 70.4, 63.1, 21.0, 20.8, 20.4.

UV (ethanol): pH 2 λ_{max} 306 nm (ε=3500), 247 nm (ε=13700); pH 7 λ_{max} 304 nm (ε=3700), 247 nm (ε=14400); pH 13 λ_{max} 295 nm (sh) (ε=4800), 274 nm (sh) (ε=8300), 248 nm (ε=15200).

Mass spectroscopy: $M^{\cdot+}$+1 at *m/z* 493 (100 %).

5-(4-Chlorobenzene)sulfenyl-2′,3′,5′-tri-O-acetyluridine (3c). 1 (0.48 g, 1.3 mmol) and 4-chlorobenzenesulfenyl chloride (0.36g, 2.0 mmol) were reacted together following the same procedure as in *3a*. The reaction was complete after 24 h with formation of two products which were characterized as 5-chloro-2′,3′,5′-tri-*O*-acetyluridine (0.04 g, 8 %) and the title compound (0.49 g, 74 %); R_f=0.51.

^{1}H NMR δ 7.96 (*s*, 1 H) H-6; 7.28 (*bs*, 4 H) aromatic protons; 6.08 (d, 4.8,1 H) H-1′; 5.36 (*m*, 2 H) H-2′ and −3′; 4.36 (*bs*, 3 H) H-4′ and −5′; 2.13 (*s*, 9 H) acetyl groups.

^{13}C NMR: δ 170.2, 169.7, 160.9, 150.2, 144.5, 133.3, 132.9, 129.8, 129.3, 107.9, 87.6, 80.3, 73.1, 70.2, 63.0, 20.8, 20.4.

UV (ethanol): pH 2 λ_{max} 300 nm (sh) (ε=4300), 253 nm (ε=16700); pH 7 λ_{max} 300 nm (sh) (ε=4400), 253 nm (ε=17400); pH 13 λ_{max} 276 nm (sh) (ε=8400), 255 nm (ε=14600).

Mass spectroscopy: $M^{\cdot+}$+1 at *m/z* 513 (32 %).

5-(4-Nitrobenzene)sulfenyl-2′,3′,5′-tri-O-acetyluridine (3d). 1 (1.0 g, 2.7 mmol) and freshly prepared 4-nitrobenzenesulfenyl chloride (1.9g, 10 mmol) were reacted together using the same conditions as in *3a*. The reaction was complete after 20 h and was followed by a work up and purification identical to that of *3a*.

Yield 0.90 g (64 %); R_f 0.47.

^{1}H NMR: δ 8.12 (*d*, 9.6, 2 H) H-3 and -5 of aromatic system; 8.10 (s, 1 H) H-6; 7.34 (d, 9.6, 2 H) H-2 and -6 of aromatic system; 6.09 (*d*, 3.6, 1 H) H-1′; 5.39 (m, 2 H) H-2′ and −3′; 4.38 (*bs*, 3 H) H-4′ and −5′; 2.13 (*s*, 3 H), 2.11 (*s*, 3 H) and 2.07 (*s*, 3 H) acetyl groups.

^{13}C NMR δ 170.2, 169.9, 169.7, 160.9, 150.2, 146.9, 145.7, 145.5, 126.2, 124.0, 104.3, 88.2, 80.3, 73.4, 70.0, 62.9, 20.6, 20.3, 20.0.

UV (ethanol): pH 2 λ_{max} 325 nm (ε=13200), 262 nm (ε=8600); pH 7 λ_{max} 326 nm (ε=13800), 262 nm (ε=8600); pH 13 λ_{max} 345 nm (ε=11500), 277 (ε=7600) Mass spectroscopy: $M^{\cdot+}$+1 at *m/z* 524 (9.9 %).

*3-N-(2-nitrobenzene)sulfenyl- and 3-N-(2,4-dinitrobenzene)sulfenyluridine, (*6a*) and (*6b*) respectively.* To a dry pyridine (10 ml/mmol) solution of uridine (*5*), trimethylchlorosilane (10 eq.) was added and the reaction mixture was stirred for 10 min; arenesulfenyl chloride (2 eq.) was subsequently added and the reaction mixture was stirred for a further period of 90 min. Methanol (2 ml/mmol) was added and the stirring was continued for 1–2 h more until formation of a low R_f product was complete. All solvents were removed; the mixture was coevporated with toluene to remove pyridine and then the title compounds were precipiteated from diethyl ether.

*Compound (*6a*).* Yield 88 %; ^{1}H NMR (DMSO-d_6): δ 8.42 (*dd*, 8.4 and 0.3, 1 H) H-3 of Dnbs group; 8.19 (*d*, 8.2, 1 H) H-6; 7.92–7.12 (*m*, 3H) H-4, -5, -6 of Nbs; 5.98 (*d*, 8.2, 1 H) H-5; 5.78 (*d*, 3.6, 1 H) H-1′; 4.03 (*m*, 3 H) H-2′, -3′ and -4′; 3.66 (*m*, 2 H) H-5′,

UV (ethanol): pH 2 λ_{max} 360 nm (ε=1800), 257 nm (ε=12400), 246 nm (ε=12800); pH 7 λ_{max} 358 nm (ε=2700), 257 nm (ε=12600), 246 nm (ε=13100); pH 13 λ_{max} 368 nm (ε=1700), 246 nm (ε=12300).

*Compound (*6b*).* Yield 93 %; UV (ethanol): pH 2 λ_{max} 313 nm (ε=9510), 266 nm (ε=16300); pH 7 λ_{max} 314 (ε=10000), 267 nm (ε=17100); pH 13 λ_{max} 336 nm (ε=6500), 259 nm (ε=13500) ^{1}H NMR (DMSO-d_6): δ 8.96 (*d*, 2.4, 1 H) H-3 of Dnbs; 8.43 (*dd*, 9.6 and 2.5, 1 H) H-5 of Dnbs; 8.23 (*d*, 8.4, 1 H) H-6; 7.54 (*d*, 9.0, 1 H) H-6 of Dnbs; 5.99 (*d*, 8.5, 1 H) H-5; 5.78 (*d*, 3.6, 1 H) H-1′; 4.0 (*m*, 3 H) H-2′, -3′ and -4′; 3.66 (*m*, 2 H) H-5′.

*Preparation of 3′,5′-O-*1,1,3,3-tetraisopropyldisiloxyl-3-N-*(2-nitrobenzene)sulfenyl uridine (*7a*) and 3′,5′-O-*1,1,3,3-tetraisopropyldisiloxyl-3-N-*(2,4-dinitrobenzene)-sulfenyluridine (*7b*). A general one-pot procedure:* To a dry pyridine solution (10 ml/mmol) of uridine, 1,1,3,3-tetraisopropyl-1,3-dichlorosiloxane (1.15 eq.) was added. The reaction mixture was

stirred for 1 h at 20 °C. Trimethylchlorosilane was then added to the reaction mixture, and after 10 min the arenesulfenyl chloride (2 eq.) was added. The reaction was then allowed to proceed for 1–2 h. Then the mixture was poured into a saturated solution of sodium bicarbonate (ca. 100 ml) and extracted with chloroform (3×50 ml). The organic layers were pooled and evaporated to dryness by coevaporations with toluene. The residue was dissolved in a 2 % ethanol-chloroform mixture, 4-toluenesulfonic acid monohydrate (1 eq.) was added and the reaction stirred for 1 min at room temperature, then poured into a saturated solution of sodium bicarbonate and extracted with chloroform. The organic layers were pooled, evaporated to dryness and purified by silica gel column chromatography, using dichloromethane as eluent.

*Compound (*7a*)*. Yield 83 %. ^{1}H NMR: δ 8.35 (*dd,* 6.6 and 0.3, 1 H) H-3 of Nbs group; 7.84 (*d,* 8.4, 1 H) H-6; 7.59–6.83 (*m,* 3 H) H-4, -5 and -6 of Nbs; 5.90 (*d,* 8.4, 1 H) H-5; 5.77 (*s,* 1 H) H-1′; 4.15 (*m,* 5 H) H-2′, -3′, -4′ and -5′; 1.08 (*m,* 28H) isopropyl groups.

UV (ethanol): pH 2 λ_{max} 360 nm (ε=2900), 260 nm (ε=13 100), 246 nm (ε=14 300); pH 7 λ_{max} 359 nm (ε=2900), 260 nm (ε=13 700), 246 nm (ε=14 500); pH 13 λ_{max} 392 nm (ε=1400), 247 nm (ε=13 100).

*Compound: (*7b*)*. Yield 77 %. ^{1}H NMR: δ 9.16 (*d,* 2.4, 1 H) H-3 of Dnbs; 8.38 (*dd,* 9.0 and 2.4, 1 H) H-5 of Dnbs; 7.90 (*d,* 7.8, 1 H) H-6; 7.11 (*d,* 9.0, 1 H) H-6 of Dnbs; 5.71 (*s,* 1 H) H-1′; 4.17 (*m,* 5 H) H-2′, -3′ and -5′; 1.02 (*m,* 28 H) isopropyl groups, UV (ethanol): pH 2 λ_{max} 315 nm (ε=8900), 266 nm (ε=15 600); pH 7 λ_{max} 314 nm (ε=9600), 266 nm (ε=16 100); pH 13 λ_{max} 336 nm (ε=6400), 259 nm (ε=11 300).

*Preparation of 3′,5′-O-*1,1,3,3-tetraisopropyldisiloxyl-2′-O-*methyl-3-*N-*(2-nitrobenzene) sulfenyluridine (*8a*) and 3′,5′-O-*1,1,3,3-tetraisopropyldisiloxyl-2′-O-*methyl-(2,4-dinitrobenzene)sulfenyluridine (*8b*). Compound 3a* or *3b* was coevaporated with toluene and then dissolved in dry acetone (10 ml/mmol). Iodomethane (10 eq.) and silver oxide (8 eq.) were added, and the reaction mixture was stirred for 16 h at 20 °C, by which time TLC showed complete reaction had occurred. The mixture was filtered, evaporated to dryness and purified by silica gel chromatography using dichloromethane as eluent.

*Compound: (*8a*)*. Yield 94 %. ^{1}H NMR: δ 8.37 (*dd,* 9.0 and 0.3, 1 H) H-3 of Nbs; 8.11 (*d,* 8.4, 1 H) H-6; 7.89–6.85 (*m,* 3 H) H-4, -5 and -6 of Nbs; 5.90 (*d,* 8.4, 1 H) H-5; 5.79 (*s,* 1 H) H-1′; 4.21 (*m,* 4 H) H-3′, -4′ and -5′; 4.09 (*bs,* 1 H) H-2′; 3.64 (*s,* 3 H) 2′-*O*-methyl; 1.11 (*m,* 28H) isopropyl groups.

UV (ethanol) pH 2 λ_{max} 366 nm (ε=1200), 266 nm (ε=6500), 244 nm (ε=6800); pH 7 λ_{max} 366 nm (ε=1200), 266 nm (ε=6800), 245 nm (ε=7200); pH 13 λ_{max} 392 nm (ε=900), 242 nm (ε=7800).

*Compound: (*8b*)*. ^{1} H NMR δ 9.16 (*d,* 2.3, 1 H) H-3 of Dnbs; 8.37 (*dd,* 8.3 and 2.3, 1 H) H-5 of Dnbs; 8.13 (*d,* 8.2, 1 H) H-6; 7.10 (*d,* 9.3, 1 H) H-6 of Dnbs; 5.90 (*d,* 8.2, 1 H) H-5; 5.75 (*s,* 1 H) H-1′; 4.19 (*m,* 4 H) H-3′, -4′ and -5′; 4.07 *bs,* H-2′; 3.62 (*s,* 3H) 2′-*O*-methyl; 1.11 (*m,* 28H) isopropyl groups.

UV (ethanol) pH 2 λ_{max} 312 nm (ε=8700), 266 nm (ε=17 300); pH 7 λ_{max} 312 nm (ε=9100), 266 nm (ε=18 100); pH 13 λ_{max} 332 nm (ε=6300), 258 nm (ε=15 000).

*Preparation of 2′-*O*-methyluridine (9). Method A.* Compound *8b* was coevaporated to dryness with toluene and dissolved in dry tetrahydrofuran (10 ml/mmol). A 1 M solution of tetrabutylammonium fluoride (2 eq.) in dry tetrahydrofuran was added to this solution and the reaction mixture stirred for 2 min at 20 °C. All the solvent was then removed by evaporation and the product (9) was isolated in 92 % yield by silica gel chromatography using an 8 % ethanol–chloroform mixture as eluent.

^{1}H NMR (DMSO-d_6): δ 7.92 (*d,* 8.4, 1 H) H-6; 5.85 (*d,* 2.4, 1 H) H-1′; 5.60 (*d,* 9.0, 1 H) H-5; 4.15 (*m,* 1 H) H-2′; 3.81 (*m,* 4 H) H-3′, -4′ and -5′; 3.37 (*s,* 3 H) 2′-*O*-methyl.

Method B. Compound *8a* was treated using the same conditions as in method A; after silica gel chromatography the title compound (*9*) was isolated in 10 % yield, and was identical to the previously prepared sample. A second compound was eluted from the chromatographic column in 81 % yield using a 6 % ethanol-chloroform mixture as eluent. This compound was identified as 2′-*O*-methyl-3-*N*-(2-nitrobenzene)sulfenyluridine.

^{1}H NMR (DMSO-d_6): δ 8.35 (*d,* 6.0, 1 H) H-3 of Nbs; 8.23 (*d,* 8.4, 1 H) H-6; 7.77–7.22 (*m,* 2 H) H-4 and -5 of Nbs; 6.95 (*d,* 7.2, 1 H) H-6 of Nbs; 5.91 (*d,* 8.4, 1 H) H-5; 5.88 (*d,* 2.4, 1 H) H-1′; 4.25 (*m,* 1 H) H-2′; 3.93 (*m,* 4 H) H-3′, -4′ and -5′; 3.58 (*s,* 3 H) 2′-*O*-methyl.

UV (ethanol) pH 2 λ_{max} 366 nm (ε=3800), 271 nm (ε=12 000), 237 nm (ε=14 100); pH 7

λ_{max} 366 nm (ε=3900), 270 nm (ε=12300), 239 nm (ε=14 800); pH 13 λ_{max} 396 nm (ε=3300), 240 nm (ε=15600).

This compound was co-evaporated with, and then redissolved in dry pyridine (10 ml/mmol). A 0.5 M dry acetonitrile solution of triethylammonium thiocresolate (2 eq.) was added and the reaction mixture stirred for 10 min. The reaction mixture was partitioned between chloroform and water and the organic phase was further extracted with water (3×20 ml). The combined aqueous layers were then evaporated to dryness and purified by preparative TLC, using 20 % ethanol-chloroform as eluent. The product isolated was identical to the compound obtained using method A and from a previous synthesis.[14] Yield 81 %; combined yield 91 %.

Preparation of 2'-O-(4-methoxytetrahydropyranyl)-3-N-(2-nitrobenzene)sulfenyluridine (11a) and 2'-O-(4-methoxytetrahydropyranyl)-3-N-(2,4-dinitrobenzene) sulfenyluridine (11b). 2'-*O*-(4-methoxytetrahydropyranyl)uridine (*10*) was coevaporated with and then redissolved in dry pyridine (10 ml/mmol). Trimethylchlorosilane (6 eq.) was then added and stirred for 10 min. The arenesulfenyl chloride was then added, and after a further 2 h the mixture was poured into a saturated sodium bicarbonate solution (ca 100 ml), extracted with chloroform (6×50 ml) and purified by silica gel chromatography. The trimethylsilyl group on the 3'-position showed considerable stability to methanolysis and thus the 3'-*O*-trimethylsilyl ethers of *11a* and *11b* were also isolated in 19 % and 5 % yields respectively.

*Compound (*11a*).* Yield 65 %. ^{1}H NMR (DMSO-d_6): δ 8.35 (*dd*, 7.8 and 2.6, 1 H) H-3 of Nbs; 8.09 (*d*, 8.4, 1 H) H-6; 7.73–6.85 (*m*, 3 H) H-4, -5 and -6 of Nbs; 6.08 (*d*, 4.8, 1 H) H-1'; 5.97 (*d*, 8.4, 1 H) H-5; 4.62 (*t*, 4.8, 1 H) H-2'; 4.15 (*m*, 2 H) H-3' and -4'; 3.82 (*m*, 2 H) H-5'; 3.66 (*m*, 4 H) H-2 and -6 of MTHP; 3.17 (*s*, 3 H) methoxyl of MTHP; 1.82 (*m*, 4 H) H-3 and -5 of MTHP.

UV (ethanol): pH 2 λ_{max} 366 nm (ε=4300), 268 nm (ε=13 000), 238 nm (ε=16 400); pH 7 λ_{max} 366 nm (ε=4400), 269 nm (ε=13 500), 239 nm (ε=17 000); pH 13 λ_{max} 398 nm (ε=2500), 240 nm (ε=14 600).

*Compound (*11b*).* Yield 63 %. ^{1}H NMR (DMSO-d_6): δ 8.96 (*d*, 2.4, 1 H) H-3 of Dnbs; 8.45 (*dd*, 9.6, 1 H) H-5 of Dnbs; 8.26 (*d*, 7.8, 1 H) H-6; 7.34 (*d*, 9.6, 1 H) H-6 of Dnbs; 6.09 (*d*, 8.4, 1 H) H-5; 6.03 (*d*, 6.0, 1 H) H-1'; 4.42 (*m*, 1 H) H-2'; 3.99 (*m*, 2 H) H-3' and -4'; 3.66 (*m*, 6 H) H-2 and -6 of MTHP and H-5'; 3.28 (*s*, 3 H) methoxyl of MTHP; 1.76 (*m*, 4 H) H-3 and -6 of MTHP.

UV (ethanol): pH 2 λ_{max} 314 nm (ε=12 300), 264 nm (ε=23 800); pH 7 λ_{max} 314 nm (ε=13 900), 256 nm (ε=23 200).

*Preparation of 5'-O-(9-phenylxanthen-9-yl)-2'-O-(4-methoxytetrahydropyranyl)-3-N-(2-nitrobenzene)sulfenyluridine (*12a*) and 5'-O-(9-phenylxanthen-9-yl)-2'-O-(4-methoxytetrahydropyranyl)-3-N-(2,4-dinitrobenzene)sulfenyluridine (12b).* Compound *11a* or *11b* was coevaporated with and then redissolved in dry pyridine (10 ml/mmol). 9-Chloro-9-phenylxanthene (1.1 eq) was then added and the reaction mixture was stirred for 20 min, a further aliquote (0.2 eq.) of the reagent was then added. After 20 min the mixture was poured into a saturated sodium bicarbonate solution (*ca.* 100 ml) and extracted with chloroform (3×40 ml). The organic extracts were combined and evaporated to obtain a residue which was further coevaporated with toluene and purified by silica gel column chromatography using a 4 % ethanol-chloroform mixture as eluent.

*Compound (*12a*).* Yield 88 %. ^{1}H NMR; δ 8.35 (*d*, 7.8, 1 H) H-3 of Nbs; 8.07 (*d*, 8.4, 1 H) H-6; 7.34 (*m*, 16 H) H-4, -5 and -6 of Nbs and pixyl group; 6.19 (*d*, 6.0, 1 H) H-1'; 5.76 (*d*, 8.4, 1 H) H-5; 4.68 (*m*, 1 H) H-2'; 4.15 (*m*, 4 H) H-3', -4' and -5'; 3.70 (*m*, 4 H) H-2 and -6 of MTHP; 3.24 (*s*, 5 H) methoxyl of MTHP and H-5'; 1.86 (*m*, 4 H) H-3 and -5 of MTHP.

*Compound (*12b*).* Yield 89 %. ^{1}H NMR: δ 9.16 (*d*, 1.8, 1 H) H-3 of Dnbs; 8.37 (*dd*, 9.0 and 1.8, 1 H) H-5 of Dnbs; 8.13 (*d*, 8.4, 1 H) H-6; 7.34–7.01 (*m*, 14 H) H-6 of Dnbs and pixyl group; 6.15 (*d*, 4.8, 1 H) H-1'; 5.75 (*d*, 8.4, 1 H) H-5; 4.66 (*m*, 1 H) H-2'; 4.15 (*m*, 2 H) H-3' and -4'; 3.70 (*m*, 4 H) H-2 and -6 of MTHP; 3.24 (*s*, 5 H) methoxyl of MTHP and H-5'; 1.86 (*m*, 4H) H-3 and -5 of MTHP.

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