175. Synthesis and Properties of Some Spin Labeled Uridine and 2′-Deoxyuridine Analogues[1])

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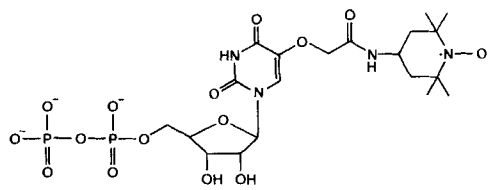
Summary

The synthesis of the spin-labeled uridine and 2′-deoxyuridine analogues RUGT **2**, DUGT **3**, *l*-RUGT **4**, and *l*-DUGT **5** is described. DUGT **3** showed some activity against the leukemia P388 cell line.

Introduction. – Spin-labeled nucleic acids provide an effective and unique means of studying the structure-function relationships of biochemical systems by ESR. spectroscopy [1]. Due to the difficulty of achieving site-specific modification of nucleic acids, relatively few spin-labeled polynucleotides have been prepared [2]. Several non-site-specific chemically modified polynucleotides [1] have been used for several years to characterize conformational transitions of nucleic acids and protein-nucleic acid interactions [3] [4]. The enzymatic incorporation of the spin-labeled nucleotide ppRUGT **1** into polynucleotides [2] was the first such synthesis of a well-defined spin-labeled polynucleotide, and has proven to be of general utility in the study of biochemical systems.

The detailed synthesis[3]) of the spin-labeled nucleosides ribouracilglycosyl-amidotempo (RUGT, **2**) and deoxyribouracilglycosylamidotempo (DUGT, **3**)[4])



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[2]) To whom reprint requests should be addressed.

[3]) A partial description of the synthesis of **2** and **4** was previously reported [2].

[4]) RUGT, *N*-[1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl]-*O*-[1-*β*-D-ribofuranosyluracil-5-yl]-glycolamide; DUGT,*N*-[1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl]-*O*-[*β*-D-2′-deoxyribofuranosyluracil-5-yl]-glycolamide.

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and of the di-labeled nucleosides, *N*(3)-spin-labeled RUGT (*l*-RUGT, **4**) and *N*(3)-spin-labeled DUGT (*l*-DUGT, **5**) is described herein. The synthetic approach is reaction of 5-hydroxyuridine **6** and of 5-hydroxy-2′-deoxyuridine (**7**) with 4-(*α*-iodoacetamido)-2,2,6,6-tetramethyl-*N*-oxido-piperidyl (**8b**). Also observed as products of the syntheses are the doubly substituted derivatives **4** and **5**.

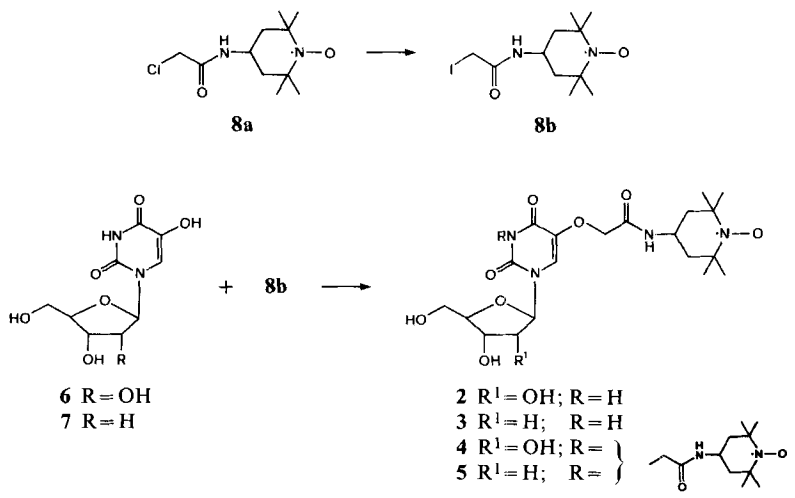
Of additional interest, it has been shown that numerous 5-substituted derivatives of 2′-deoxyuridine exhibit inhibition of viral cytopathogenicity, in contrast to 5-substituted uridine analogues [5]. The results of cytotoxicity tests of compounds **2** and **3** in several transformed cell lines are also reported here.

Results. – For the effective substitution of the nucleosides, 4-(*α*-chloroacetamido)-2,2,6,6-tetramethyl-*N*-oxido-piperidyl (**8a**) was converted into the more reactive **8b** by treatment of **8a** with 1.1 mol-equiv. of sodium iodide in anhydrous acetone. *Scheme 1* summarizes the results obtained from the reaction of **6** and **7** with **8b**. When either **6** or **7** was treated first with base in a buffered medium, and then with 1 mol-equiv. of **8b**, two main products were isolated by preparative thin-layer chromatography in both cases.

The reaction of **6** with the activated piperidyl radical yielded the 5-*O*-substituted ribonucleoside **2** (Rf 0.56) and the di-substituted ribonucleoside **4** (Rf 0.63). The UV. spectra of **2** (275 nm, $\varepsilon = 7600$, pH 7.0), and of **4** (275 nm, $\varepsilon = 8000$, pH 7.0), together with the ^{1}H-NMR. spectra, support these conclusions.

Since the NMR. spectra of free radical compounds are generally unresolved [6], the spectra of **2** and **4** were obtained after reduction of the piperidyl radical to the

Scheme 1



Scheme 2

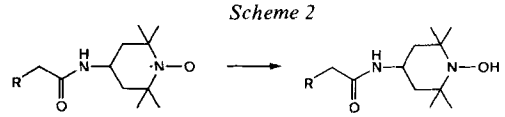


Table 1. *Assignments of relevant H-atoms in the ^{1}H-NMR. spectra.* (s = singlet, m = multiplet)

Compound	HO–C(5)	H–C(6)	H–N(3)	CH_3 (piperidine)	CH_2 (piperidine)	CH_2 (glycolamide)	NH (glycolamide)
6	8.4 (*s*)	7.35 (*s*)	11.6 (*s*)	–	–	–	–
7	8.4 (*s*)	7.37 (*s*)	11.6 (*s*)	–	–	–	–
2	–	7.80 (*s*)	11.60 (*s*)	1.40 (*s*)	1.80 (*m*)	4.40 (*s*)	8.20 (*m*)
3	–	7.70 (*s*)	11.50 (*s*)	1.40 (*s*)	1.80 (*m*)	4.30 (*s*)	8.20 (*m*)
4	–	7.96 (*s*)	–	1.40 (*s*)	1.80 (*m*)	4.40 (*m*)	8.30 (*m*)
5	–	7.80 (*s*)	–	1.40 (*s*)	1.80 (*m*)	4.40 (*m*)	8.20 (*m*)

corresponding hydroxylamine derivative with $Na_2S_2O_4$ *(Scheme 2)*, followed by transfer of the reduced species to DMSO-d_6[5]). The ^{1}H-NMR. spectrum of **2**, compared with that of **6** *(Table 1)*, showed that the HO–C(5) signal of **6** had vanished, and that the H–C(6) singlet had shifted downfield from the value for **6**. These results, with the indication of 4 equivalent methyl groups, supported structure **2**. The spectrum of **4** *(Tab. 1)* showed that both the HO–C(5) and the H–N(3) signals of **6** had vanished, and together with the indication of 8 methyl groups, supported the disubstituted structure **4**.

The reaction of **7** with **8b** under the same conditions as for **6** yielded two analogous products, the 5-*O*-substituted deoxyribonucleoside **3** (Rf 0.57), and the disubstituted deoxyribonucleoside **5** (Rf 0.64). The UV. spectra of **3** (275 nm, $\varepsilon = 7600$, pH 7.0) and of **5** (275 nm, $\varepsilon = 7900$, pH 7.0) and their respective ^{1}H-NMR. spectra corroborated these structures.

The ^{1}H-NMR. spectra of **3** and **5** also were obtained following reduction with $Na_2S_2O_4$ *(Scheme 2)* and then transfer of the reduced materials to DMSO-d_6, and are analogous to those of **2** and **4**, respectively. Comparison of the ^{1}H-NMR. spectrum of **3** with that of **7** *(Table 1)* showed that the HO–C(5) signal of **7** had vanished, the H–C(6) singlet had shifted downfield from the value for **7**, and that 4 equivalent methyl groups were indicated. The spectrum of **5** showed the absence of both the HO–C(5) and the H–N(3) signals of **7**, and indicated 8 methyl groups.

Biological activity[6]). – The cytotoxicities of compounds **2** and **3** were evaluated in three different cell lines – leukemia P388, leukemia L1210, and in lymphoma L5178Y. The results in *Table 2* show that compound **3** was active against leukemia

Table 2. *The cytotoxicities of RUGT and DUGT in the Leukemia P388 Cell Line*

Dose, μg/ml	% Inhibition			
	Compound **2**		Compound **3**	
	24 h	48 h	24 h	48 h
100	0	0	40	76
10	0	0	2	1
1	0	1	2	0
0.1	0	0	0	0
0.01	0	0	0	0

[5]) Full details of the method are forthcoming.

[6]) We thank Dr. *R. H. Adamson, National Cancer Institute, NIH,* for the biological activity studies.

P388 at a dose level of 100 μg/ml, whereas compound **2** was inactive. Compound **3** exhibited much less activity against leukemia L1210 and lymphoma L5178Y, and compound **2** again showed no activity against these cell lines.

Experimental part

General. The melting points were determined with a Mel-Temp and are uncorrected. pH was monitored with a *Beckman* Expandomatic SS-2 pH meter. The UV. spectra were measured with a *Cary*-14 spectrophotometer (ε values ± 3%). The 60-MHz-^{1}H-NMR. spectra were monitored with a *Varian* T60 spectrometer using TMS as the internal standard. A *Büchler* flash-evaporator was used for solvent removal.

The spin-label **8a** was purchased from *Eastman* (Rochester, New York), and the preparations of **6** [7] and **7** [8] were slightly modified from the literature. Preparative TLC. was done with silica gel GF. plates purchased from *Analtech* (Newark, Delaware) ($CHCl_3$/MeOH 41:9).

1. *5-Hydroxyuridine* (**6**) from uridine. To a stirred solution of 1.0 g of uridine in 40 ml of H_2O at RT. saturated aqueous bromine-solution was added dropwise until a light yellow color persisted (approximately 35 ml). Filtered air was bubbled through the solution until it became colorless. To the clear solution was added 30 ml of pyridine dropwise so that the temperature was not increased. After 18–24 h at RT., the mixture was evaporated under vacuum below 30° to a viscous syrup. This was dissolved in 40 ml of abs. ethanol at 50° and filtered hot. The filtrate was concentrated to one-third of the original volume, equilibrated to RT., then cooled to 4° overnight. The crystals were filtered off and washed with abs. ethanol. Recrystallization from 90% ethanol at 50° gave 520 mg of **6**, m.p. 223°. A second recrystallization gave translucent crystals, m.p. 239–240°. Compound **6** gave a blue color with saturated aqueous $FeCl_3$, and was unstable in alkali. – UV.: $\lambda_{max}^{pH\,2}$ 279 nm (ε 8300); $\lambda_{max}^{pH\,7}$ 281 nm (ε 7500); $\lambda_{max}^{pH\,12}$ 303. – ^{1}H-NMR. (DMSO-d_6): see *Table 1.*

2. *5-Hydroxy-2′-deoxyuridine* (**7**) from 2′-deoxyuridine. The procedure was similar to that for the preparation of **6**, starting with 1.0 g of 2′-deoxyuridine. Subzero temperatures were used after induction of crystallization at 4°, and isolation of the crystals was done in a cold room, yielding 420 mg of **7**. Two recrystallizations from 90% ethanol (50°) gave **7**, m.p. 211–213°. Compound **7** gave a blue color with saturated aqueous $FeCl_3$, and was unstable in alkali. – UV.: $\lambda_{max}^{pH\,2}$ 280 nm (ε 8000); $\lambda_{max}^{pH\,7}$ 281 nm (ε 7100); $\lambda_{max}^{pH\,12}$ 303 nm. – ^{1}H-NMR. (DMSO-d_6): see *Table 1.*

3. *4-(α-Iodoacetamido)-2,2,6,6-tetramethyl-N-oxido-piperidinyl* (**8b**) *from* **8a**. A solution of 150 mg of **8a** in 2.0 ml of anhydrous acetone (dried over 4 Å molecular sieves) was treated with 113 mg of NaI. The mixture was stirred overnight in a sealed vial at RT. The solid KCl was centrifuged out, and the clear, orange supernatant containing **8b** was directly used in the preparation of **2**, **3**, **4** and **5**.

4. *RUGT* (**2**) *and l-RUGT* (**4**) *from* **6**. To a solution of 150 mg of **6** in 1.4 ml of H_2O was added 0.58 ml of 1N KOH, and 0.50 ml of 0.5M potassium phosphate buffer, pH 7.8. The reagent **8b** was added dropwise to this solution. The resulting mixture (pH 9.3) was sealed and stirred at 37°. After 2 h, 0.30 ml of 1N KOH was added, resulting in pH 9.7. Stirring at 37° was resumed. After 3 h the addition of 0.10 ml of 1N KOH resulted in pH 9.3, and the mixture was stirred at 37° for 40 h. The addition of 0.05 ml of 1N KOH resulted in pH 8.8. Constant pH after 2 h of stirring indicated complete reaction. The solvents were removed under diminished pressure, and the orange-yellow gum was dissolved in 1–2 ml of abs. ethanol. The crude mixture was separated by preparative TLC., the fifth and sixth bands from the origin yielding 76 mg of **2** (contaminated with **4**), and 28 mg of **4**, respectively. The crude product **2** on rechromatography gave 25–30 mg of **2**.

Compound **2**: Amorphous orange powder, negative to $FeCl_3$, m.p. 131–133°. – UV.: $\lambda_{max}^{pH\,7}$ 275 nm (ε 7600); $\lambda_{max}^{pH\,12}$ 273 nm (ε 6300). – ^{1}H-NMR. (DMSO-d_6): see *Table 1.* – Compound **2** showed a 1:1 ratio of spin-label to nucleoside by ESR.

Compound **4**: Amorphous orange powder, negative to $FeCl_3$, unstable in alkali, m.p. 153–154°. – UV.: $\lambda_{max}^{pH\,7}$ 275 nm (ε 8000). – ^{1}H-NMR. (DMSO-d_6): see *Table 1.* A dilute solution of **4** showed spin exchange *via* ESR.

5. *DUGT* (**3**) *and l-DUGT* (**5**) *from* **7**. The synthesis and isolation of **3** and **5** were achieved with the procedure described for the preparation of **2** and **4**, starting with 150 mg of **7**. The preparation gave 76 mg of **3** (contaminated with **5**), and 23 mg of **5**. Rechromatography of crude **3** gave 33 mg of **3**.

Compound **3**: Amorphous orange powder, negative to $FeCl_3$, and unstable in alkali, m.p. 121–123°. – UV.: $\lambda_{max}^{pH\,7}$ 275 nm (ε 7600); $\lambda_{max}^{pH\,12}$ 273 nm (ε 6600). – ^{1}H-NMR. (DMSO-d_6): see *Table 1*.

Compound **5**: Amorphous orange powder, negative to $FeCl_3$, and unstable in alkali, m.p. 145–147°. – UV.: $\lambda_{max}^{pH\,7}$ 275 nm (ε 7900). – ^{1}H-NMR. (DMSO-d_6): see *Table 1*.

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