



NMR studies on 4-thio-5-furan- and 4-thio-5-thiophene-modified Nucleosides

Journal:	<i>Magnetic Resonance in Chemistry</i>
Manuscript ID	Draft
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Zhang, Xiaohui; Dalian University, College of Environment and Chemical Engineering Xu, Yao; the Open University, Life, Health and Chemical Sciences
Keywords:	NMR, ¹³ C, ¹ H, 4-thiothymidine, 5-ringed nucleoside, 5-thiophene-4-thiouridine, 5-furan-4-thiouridine, modified nucleoside, DNA, x-ray

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NMR studies on 4-thio-5-furan- and 4-thio-5-thiophene-modified nucleosides

Xiao-Hui Zhang^{a*} and Yao-Zhong Xu^{b*},

^aCollege of Environment and Chemical Engineering, Dalian University Dalian 116622, China

^b Department of Life, Health and Chemical Sciences, the Open University, Walton Hall, Milton Keynes, MK7 6AA, UK

1. Introduction

Nucleosides are the key molecules playing the fundamental roles in many biological processes, including cellular survival and growth. Modified nucleoside have been designed to understand and to elucidate these processes and consequently used as potential drugs because of their structural similarity to natural ones.¹⁻² We have been working on designing and developing 4-thiothymidines as effective anti-cancer agents in combination with UVA irradiation. Previously we reported 4-thiothymidine^{3,4} and its 5-bromo-⁵ and 5-iodo-deoxyuridine⁶ as potential anti-tumor drugs.^{7,8,9} More recently we worked on such thio-analogues by conjugating 4-thiothymine with an aromatic ring, such as furan and thiophene, to shift their UVA absorption towards longer wavelengths to enhance the sensitivity to UVA irradiation.

In this paper, we report our findings from a systematic NMR study of these rarely available modified nucleosides using various NMR techniques (including COSY, NOESY) and also compare these findings with those acquired by using x-ray crystallography to offer a better understanding of the structural information of these modified nucleosides in solution and in solid state. Such structural knowledge would help to appreciate how these thio-analogues would work with UVA light and ultimately to design and produce modified nucleosides as anti-cancer therapeutics.

2. Results and Discussion

2.1. Synthesis and characterization

Chemical synthesis: The target products, 4-thio-5-(furyl-2-yl/thiophen-2-yl)-pyrimidine deoxyribonucleosides (**2a** and **3a**) and 4-thio-5-(furyl-2-yl/thiophen-2-yl)-pyrimidine ribonucleosides (**2b** and **3b**) were prepared from their corresponding deoxy-uridine (**1a**) and uridine (**1b**) as shown in **Scheme 1** (below). The preparation involved a 5-step synthesis (protecting the sugar hydroxyl groups; iodination at the 5-position of the base; replacing the iodo group with a ring (furan/thiophene); thioation at the 4-position of the base and finally releasing the sugar hydroxyls from the protecting groups). A detailed synthesis will be published in elsewhere.

* Corresponding author. Tel.: +86 411 8740 2378; e-mail: zhangxiaohui@dlu.edu.cn

* Corresponding author. Tel.: +44 1908 654 064; e-mail: y.z.xu@open.ac.uk.

(Scheme 1 and its legend here)

Characterization: The structures of 4-thio-5-(furyl-2-yl/thiophen-2-yl)-pyrimidine nucleosides (**2a-b** and **3a-b**) have been thoroughly characterized and confirmed by various spectrometric studies, including FTIR and HR-MS.

2.2. NMR study of compounds 2 and 3.

2.2.1. ¹H NMR

For the brevity of this paper, a modified nucleoside, 4-thio-5-thiophene-riboouridine (**3b**), is illustrated as a typical example. A section of 1D ¹H-NMR spectrum of **3b** is shown below. The protons on the nucleoside can be classed into three types: sugar, base and ring (furan/thiophene). In general chemical shifts for sugar protons are below 6 ppm, the protons from thiophene are located around 7 ppm while the proton (6-H) of the base is above 8 ppm. Their accurate assignments will be detailed accordingly.

(Figure 1 and its legend here)

a) Assignment of the sugar protons

The chemical shift of 1'-H has been well established and generally located at around 6 ppm. In the case of 4-thio-5-thiophene-riboouridine (**3b**), it is found to be at 5.76 ppm, from which we can readily locate its neighboring proton (i.e. 2'-H) *via* their cross peak (Peak A, in the Left panel of Figure 2). By following the arrow, the 2'-H would lead us to its cross peak (Peak B) with 2'-OH.

The carbon at 5'-position has two bonded protons which are the same chemically, but non-identical magnetically. As this set of protons is unique in the whole molecule and can be easily identified as the pair at the region below 4 ppm (more accurately at 3.56 and 3.72 ppm, see the right end of Figure 1 and the top right corner in the left panel of Figure 2). So we can start from here to trace its neighboring protons *via* peak C (crossing with 4'-H) and peak D (crossing with 5'-OH). The remaining signals for 3'-OH and 3'-H can be identified *via* their cross peak E. These assignments are further supported by the following lines of evidence: a) all three OH signals disappeared during D₂O exchange, b) the cross peaks between 2'-H and 3'-H and between 3'-H and 4'-H are identifiable in the range of 4 ppm.

(Figure 2 and its legend here)

The chemical shifts for the deoxy-sugar protons of 4-thio-5-thiophene-deoxyriboouridine (**3a**) have an identical pattern except 2'-H has a much lower δ value since its attached carbon-2 is not bonded directly to the electron-withdrawing O atom.

1
2
3 *b) Assignment of the base protons (N₃-H and 6-H)*

4 There are only two protons on the base of the 5-substituted nucleosides (e.g. **2** and
5 **3**). (See Scheme 1 for the chemical structures). One is an imino proton (N₃-H, at
6 N3-position) and can be found at the far left end (lowest field) of the ¹H NMR
7 spectrum. The chemical shift of N₃-H in **3b** is found to be at 12.89 ppm and
8 noticeably higher than those of the equivalent imino protons of un-thiolated thymine
9 bases (usually between 11 and 12 ppm).^{10,11,12} This increased δ value can be ascribed
10 to the presence of sulfur at the 4-position of the molecule (**3b**). The imino proton is
11 exchangeable, so this type of NMR signal has been verified by D₂O exchange. The
12 other proton on the base is 6-H. As it is bonded with the carbon within the
13 electron-rich and conjugated base, its chemical shift can be found at the relatively
14 lower field (8.51 ppm). This assignment has been also confirmed by 2D (¹H-¹³C)
15 spectrum (data not shown).
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21 *c) Assignment of the protons in the 5-membered rings (thiophene and furan).*

22 The chemical structure of C-5-linked thiophene is shown in the left panel of Figure
23 3. In the ring there are three protons, of which 4''-H is the only proton neighbored
24 (thus closely coupled) with *two* protons (i.e. 3''-H and 5''-H). So the signal at 7 ppm,
25 which has cross peaks with *both* 3'' and 5''-protons, can be tentatively assigned as
26 4''-H (shown in the right panel of Figure 3). The signal located at 7.5 ppm is in the
27 lowest field among these three protons and so can be putatively assigned as 5''-H, as it
28 is close to the sulfur atom. From 5''-H, we can find its cross peak with 4''-H (**F**) and
29 then track to 4''-H. From there, we can identify another cross peak (**G**), leading to the
30 remaining proton (i.e. 3''-H). In addition ¹H-¹³C COSY spectrum was also used to
31 provide further supports to the assignment of these three ring protons (see the section
32 of ¹³C NMR assignment below).
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36

37 **(Figure 3 and its legend here)**

38
39 The chemical shifts for the proton at the furan ring of 4-thio-5-furan-ribose
40 (**2b**) have a very similar pattern to those of the thiophene analogue (**3b**) except 5''-H
41 has a higher δ value as the proton at 5''-position is close to the electron-withdrawing
42 O atom in **2b**. For the related deoxynucleoside analogues (**2a** and **3a**), the NMR data
43 on these 5-membered rings (furan and thiophene) are very similar, and all ¹H NMR
44 peaks for the target nucleosides (**2** and **3**) have been identified and the data are listed
45 in Table 1.
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48

49 **(Table 1 here)**

50
51 For comparison, **Table 1** also includes ¹H NMR data^{13,14} of 5-(furyl-2-yl)-
52 deoxyribose (here named as **4a**) and 5-(furyl-2-yl)-ribose (**4b**). Compounds
53 **4a** and **4b** are the 4-oxy analogues to compounds **2a** and **2b** (i.e.
54 4-thio-5-(furyl-2-yl)-deoxyribose and 4-thio-5-(furyl-2-yl)-ribose. By
55 comparison of the data of **4a/4b** with those of **2a/2b**, it is clear both assignments agree
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2
3 very well. The only difference is on the N₃-H proton (see the highlighted values in
4 Table 1). This is because **4a/4b** have an oxygen atom at the 4-position while **2a/2b**
5 contain a sulfur atom at the same position. The presence of sulfur at the 4-position
6 shifts its neighbouring N₃-H to a lower field (i.e. a higher δ value), consistent with our
7 early observations^{11, 12}
8
9

10 2.2.2. ¹³C NMR

11 As ¹³C NMR would provide complimentary and additional structural information to
12 ¹H NMR, thus we also carried out a systemic study of these modified nucleosides.
13 Our approach was first to generate a complete 1D ¹³C spectrum of one of our
14 modified nucleosides and then assign each of the signals with the aid of other
15 techniques. Again we use 4-thio-5-thiophene-ribouridine (**3b**) as an example. A
16 complete 1D ¹³C spectrum (**Fig. S1**) is shown in electronic supplementary info (ESI).
17 The assignments of these peaks are discussed individually in each of the three
18 individual parts (sugar, base and the ring) as illustrated below.
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23 a) Assignment of the sugar carbons

24 The above-described ¹H-NMR data can be used as a starting point for assigning ¹³C
25 NMR signals, thus it is feasible to trace their corresponding carbons from the
26 identified protons. This was exemplified by ¹H-¹³C 2D NMR of **3b**. The sugar section is
27 shown in Figure 4 (for the full spectrum, see **Fig. S2** in ESI).
28
29
30

31 It is straightforward to find out from the section of ¹H-¹³C 2D NMR (Figure 4) that
32 1'-H has its corresponding ¹³C signal at near 90 ppm that is the signal for the carbon
33 at 1'-position (ie. C-1'), see the dotted line in Figure 4. Similarly for other sugar
34 protons (i.e. 2'-H, 3'-H, 4'-H and 5'-H), the chemical shifts of the related carbons can
35 be clearly identified from Figure 4 and listed in Table 2. It is worth noting that the
36 chemical shifts for C-1' carbons in the ribo-sugar (eg. **2b** and **3b**) are always higher
37 than those of C-4' carbons while in the deoxyribose sugar (eg. **2a** and **3a**) the
38 chemical shifts for C-1' are lower than those of C-4', see Table 2. This generality was
39 also noted in our early work.¹² This can be ascribed to the fact the presence of oxygen
40 atom at the 2'-position (of ribosugar) has a stronger effect on its directly neighbouring
41 C-1' carbon than its slightly distant C-4' carbon.
42
43
44
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46 **(Figure 4 and its legend here)**

47 b) Assignment of the base carbons

48 The pyrimidine base has four carbons (C-2, C4, C-5 and C-6), of which only C-6
49 has a bonded H atom. Thus the signal for C-6 can be readily identified from the
50 known 6-H *via* ¹H-¹³C 2D NMR (HMQC technique). Taking **3b** again as an example,
51 as the peak of singlet (δ 8.51 ppm) has been previously assigned as 6-H (cf. Table 1),
52 the 6-H peak can be traced in the HMQC spectrum to identify a cross-peak leading to
53 its coupled carbon at 135.77ppm. (see **Fig. S2** in ESI). However the other three
54 carbons (C-2, C-4 and C-5) are all quaternary carbons (without bonded hydrogens).
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Thus, HMQC is no use in this instance. HMBC (heteronuclear multiple bond correlation) is a possible means; the only non-exchangeable proton in the base is at the 6-position and is of three bond distance to both C-2 and C-4 and of two bond distance to C-5. So HMBC could be used to identify C-5 among them, but is unlikely to provide a conclusive solution to distinguish between 2-C and 4-C. Distortionless Enhancement by Polarization Transfer (DEPT) is a useful technique, which can allow us to determine multiplicity of carbon atoms substitution with hydrogens. As those carbon atoms without hydrogen attached, namely quaternary carbons, would become invisible in a DEPT spectrum. By comparing 1D ^{13}C NMR of **3b** and its DEPT-135 (see **Fig. S3a** and **S3b** in ESI), it is easy to see that the peaks at 187 ppm (C-4), 147 ppm (C-2), 137 ppm (C-2'') and 118 ppm (C-5) are missing in the DEPT-135 (Figure 5) and thus are identified as quaternary carbons. Three of them are from the base (i.e. C-2, C4 and C-5) and the other is from C-2'' of the thiophene.

(Figure 5 and its Legend here)

In our early work¹² we have demonstrated that presence of sulfur atom at the 4-position makes the chemical shift of C-4 move toward extremely low field, thus the peak with the highest δ value (187 ppm) should be assigned for C-4. C-2 is a type of urea and its δ value should be relatively higher, thus we can assign the peak at 147 ppm as C-2, and the peak at 118 ppm can be tentatively assigned for C-5 since its chemical shift is affected by the electronegativity of the substituent linked to C-5 position.¹² The remaining quaternary carbon (137pp) can thus be tentatively assigned for C-2'' of the thiophene, see below for further discussion.

3) Assignment of the ring (thiophene) carbons

The chemical structure of linked thiophene and its labelling are shown in Figure 6.

(Figure 6 and its legend here)

There are four carbon atoms (C-2'', C3'', C-4'' and C-5'') in the ring of thiophene. As each of the three carbons (C-3'', C-4'' and C-5'') has a bonded hydrogen, so they can be readily identified via ^1H - ^{13}C COSY spectrum (Figure 6). Although C-3'' ($\delta=126.2$) and C-4'' have a similar value ($\delta=126.2$) in ^{13}C NMR, however their respective values for ^1H NMR are noticeably different and thus can be assigned confidently. We have used the same approach to other target nucleosides and their ^{13}C data are listed in Table 2.

(Table 2 here)

Table 2 also includes ^{13}C NMR data¹³ of **4a** [5-(furyl-2-yl)-deoxyribose] for comparison. The assignments of **4a** are in good agreement with the assignments of **2a** (its 4-thio-analogue). Again the sole difference is on the carbon at the 4-position, see the values in bold format. It is worth mentioning that ^{13}C data for **4b** [5-(furyl-2-yl)-uridine] has been listed, not assigned.¹⁴

2.2.3. Relative orientation of the moieties

Nuclear Overhauser Effect spectroscopy (NOESY) is a useful tool to establish the correlations between nuclei that are spatially close and can provide valuable information about molecular structures. Thus, we used NOESY technique to study the structural orientation among nucleoside's moieties: the base, the ring and the sugar. Again take **3b** as an example. Its NOESY spectrum is shown in Figure 7.

(Figure 7 and its legend here)

The cross peaks in NOESY indicate the concerned nuclei are spatially close. In this case, peak **K** suggest that the proton at 6-position of the base (i.e. 6-H) is spatially close to 3''-H. Cross peaks **L** and **M** indicate 6-H is also spatially close to the protons at 2'-position (i.e 2'-H) and 3'-position (i.e. 3'-H) of the sugar. Therefore, we can propose the orientation of the thiophene is upward (parallel orientation), namely the sulfur atom is located at upper part of the ring as shown in Figure 7 insert. This model is consistent with our findings from x-ray structural studies shown at in Figure 8.

(Figure 8 and its legend here)

However the orientation for 5-furan-analogue is quite different. We were able to observe NOE effects for 6-H of the base with 2'-H and 3'-H of the sugar, but no NOE effect was observed between 6-H and 3''-H (see **Fig. S4** in ESI), implying the furan ring is downward (antiparallel orientation), namely the oxygen atom in the furan is away from the sulfur at the 4-position of the base. Again this finding is also consistent with our results from x-ray structural study of 4-thio-5-(furan)-riboiridine (**2b**) shown in **Figure 8**.

It is comforting to see this same finding obtained by NMR (from a solution state) and by x-ray crystallography (from a solid state) on the orientation of the base relative to the ring (furan/thiophene). However, lacking is the suitable explanation of why **3b** has a parallel orientation while **2b** has an antiparallel structure. Although we could attribute these orientations to the facts that the large-sized sulfur atom in **3b** has to avoid the stereo hindrance from the base and the sugar moieties, thus keeping its parallel orientation while the relatively smaller oxygen atom in **2b** does not have the necessity to do so. More work will be required to completely answer these questions.

3. Conclusions

4-thio-5-furan- and 5-thiophene-modified ribouridines and their 2'-deoxy analogues have been successfully prepared. In addition to standard analyses, their ¹H and ¹³C NMR have been systemically investigated and various NMR spectroscopic approaches are used to unambiguously assign all protons and all carbons in these novel modified nucleosides. The orientations of the base (4-thiouridine or its deoxy analogue) relative to the ring (furan or thiophene) are confirmed by both NMR and x-ray crystallographic studies.

4. Experimental Section

General procedures for the synthesis of modified nucleosides (2 and 3)

Melting point was determined on a XR-4-type micro-melting point detector without correction. The compounds synthesized were purified by column chromatography using silica gel (200–300 mesh) except for recrystallization and thin-layer chromatography (TLC) using silica gel 60 F254 plates (250 mm; Qingdao Ocean Chemical Company, China). IR spectra were recorded using a Nicolet 550 Spectrophotometer ($4000\sim 400\text{ cm}^{-1}$) with a crystalline sample spread on KBr pellets. UV spectra were recorded using UV-VIS spectrophotometer (JASCO, Japan); ^1H NMR and ^{13}C NMR spectra were obtained by a 500 MHz Bruker AV-400 spectrometer with TMS as an internal standard. The mass spectrum was obtained on Hewlett-Packard 1100 LC/MSD spectrometer.

Protecting the sugar hydroxyl groups: The nucleoside (deoxyribose, **1a** or uridine, **1b**) (4.09 mmol) dissolved in anhydrous pyridine (12 mL), was treated with dry acetic anhydride (2.75 mL, 29 mmol) for 5 h. The yields were at 97-99%.

Iodination at the 5-position of the base: A mixture of acetyl protected nucleosides (0.5 mmol), iodine (76 mg, 0.3 mmol), Ceric ammonium nitrate (137 mg, 0.25 mmol) and MeCN (8 mL) was stirred at 80°C for 1 h. The yields were around 45% -70%.

Replacing the iodo group with a ring (furan/thiophene): Bis (triphenylphosphine) palladium (II) chloride (0.016 g, 0.023 mmol) and 2-(trimethyltannyl)-furan /-thiophene (3.42 mmol) was added to the solution of 5-iodo-acetyl protected nucleosides (1.14 mmol). The mixture was heated at 90°C and refluxing for 3 h. The yields were around 75%.

Thioation at the 4-position of the base: 5-(furyl-2-yl/thiophen-2-yl)-acetyl protected nucleosides (2.32 mmol) were dissolved in peroxide-free 1,4-dioxane (50 mL) and P_2S_5 (1.0 g, 4.50 mmol) was added. The mixture was refluxed for 3-4 h. The yields were around 49-64%.

Releasing the sugar hydroxyls from the protecting groups: 4-thio-5-(furyl-2-yl/thiophen-2-yl)-acetyl protected nucleosides (2.14 mmol) were suspended in absolute MeOH (60 mL, 1.5 mmol) and saturated with dry ammonia gas. The mixture was stirred at room temperature for 4.5 h. The yields for the target products (4-thio-5-(furyl-2-yl/thiophen-2-yl) nucleosides (**2a-b** and **3a-b**) were around 45%-70%.

NMR instruments and techniques

500 MHz from Bruker (AV-500, FT NMR) and 400 MHz from Bruker (AV-400, FT NMR) were used. The COSY spectra (DMSO- d_6) were obtained in the magnitude mode with 1024 points in the F2 dimension and 256 increments in the F1 dimension. Each increment FID was obtained with 12 scans with a relaxation delay of 2 s. HMQC spectra (DMSO- d_6) were obtained in the magnitude mode with 1024 points in the F2 dimension and 256 increments in the F1 dimension. Each increment FID was obtained with 16 scans with a relaxation delay of 2 s. NOESY spectra (DMSO- d_6) experiments were obtained in the magnitude mode with 2048 points in the F2

dimension and 256 increments in the F1 dimension. Each increment FID was obtained with 16 scans with a relaxation delay of 2 s.

5. Acknowledgements

The work was supported by the Dalian government's science-technology plan projects (Grant No. 2014E12SF074), the public welfare foundation project of science enterprise investigation in Liaoning province of China (2015), and the Dalian Jinzhou New Area government's science-technology plan projects (Grant No. KJCX-ZTPY-2014-0009).

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Table 1 ¹H NMR data for all protons in furan- and thiophene-modified nucleosides

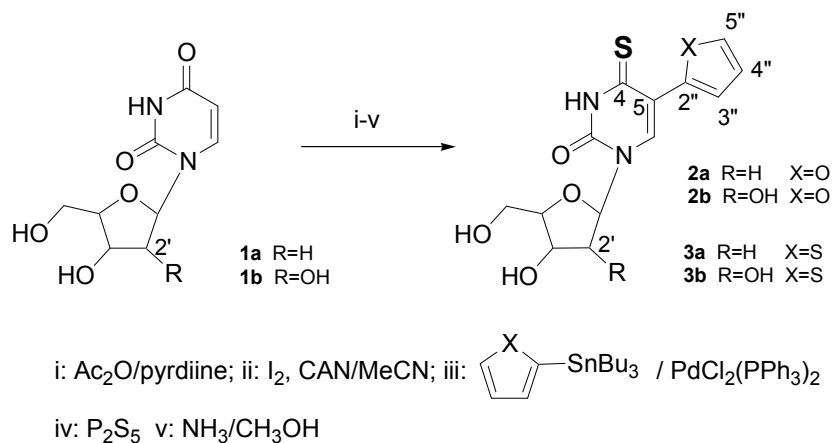
Cpds	Sugar					Base	Ring			Exchangeable			
	1'-H	2'-H	3'-H	4'-H	5'-H		3''-H	4''-H	5''-H	NH	2'-OH	3'-OH	5'-OH
2a	6.08	2.18	4.21	3.81	3.55	8.43	7.26	6.46	7.56	12.83	-	5.10	5.29
2b	5.82	4.14	4.03	3.94	3.67	8.61	7.29	6.52	7.61	12.91	5.81	5.81	5.81
3a	6.14	2.27	4.29	3.85	3.63	8.44	7.32	7.03	7.51	12.91	-	5.23	5.29
3b	5.76	4.12	4.02	3.89	3.64	8.51	7.27	6.99	7.48	12.89	5.51	5.07	5.31
4a*	6.22	2.18	4.28	3.84	3.61	8.33	6.86	6.52	7.62	11.63	-	5.27	5.08
4b*	5.86	4.13	4.00	3.90	3.68	8.42	6.86	6.53	7.61	11.66	5.43	5.11	5.20

*) Data¹³ for **4a** [5-(furyl-2yl)-2'-deoxyribouridine] and data¹⁴ for **4b** [5-(furyl-2yl)-ribouridine] are from the literature

Table 2 ^{13}C NMR data for all carbons in furan- and thiophene-modified nucleosides

Cpd	Sugar					Base				Ring			
	1'	2'	3'	4'	5'	2	4	5	6	2''	3''	4''	5''
2a	86.2	40.9	70.5	88.4	61.3	148	186	110	135	147	112	114	142
2b	89.6	74.8	69.9	85.4	60.6	148	186	110	135	147	112	114	142
3a	86.1	40.9	70.2	88.4	61.1	147	187	118	135	137	126	126	127
3b	89.6	74.8	69.9	85.4	60.6	148	188	118	136	137	126	126	127
4a*	84.7	40.1	70.4	87.6	61.1	149	160	106	135	146	108	112	141

*) The ^{13}C data and assignments of **4a** [5-(furyl-2yl)-2'-deoxyribouridine] is from literature ¹³.



Scheme 1. Synthetic routes to 4-thio-5-(furyl-2-yl/thiophen-2-yl)-pyrimidine nucleosides. The conventional numberings are used for the sugar and base. The numberings of the ring (furan or thiophene) are shown in the top right of the structures **2** and **3**.

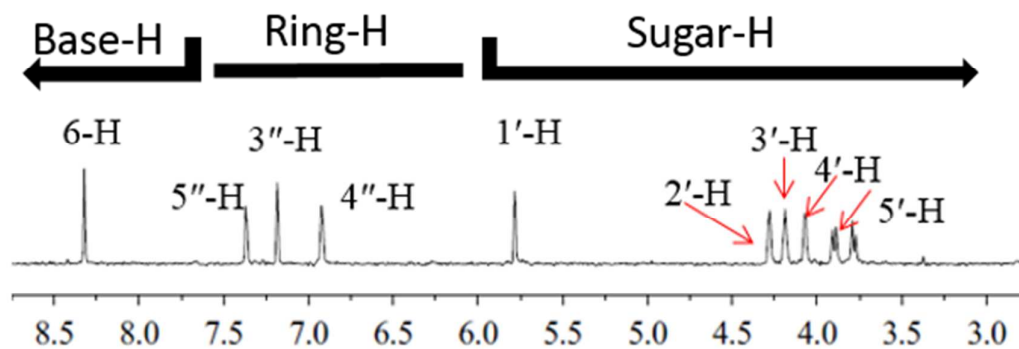


Figure 1. Section of ^1H NMR spectrum of 4-thio-5-thiophene-ribose (3b) and its assignments

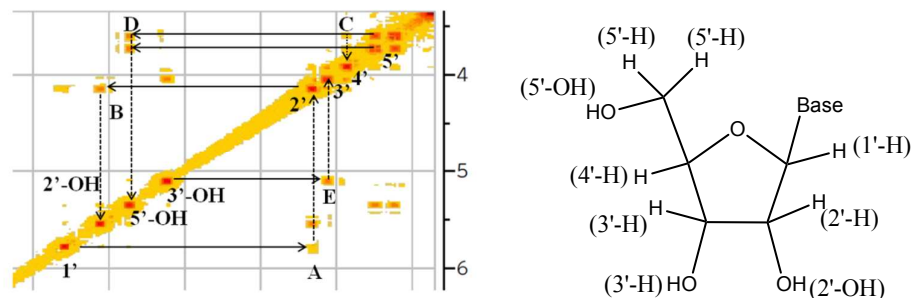


Figure 2. Left: Section of 2D ^1H - ^1H NMR of **3b**, Right: chemical structure and numberings for the sugar moiety of **3b**. The cross peaks are labelled as **A** (between 1'-H and 2'-H), **B** (between 2'-H and 2'-OH), **C** (between 4'-H and 5'-H), **D** (between 5'-H and 5'-OH) and **E** (3'-H and 3'-OH).

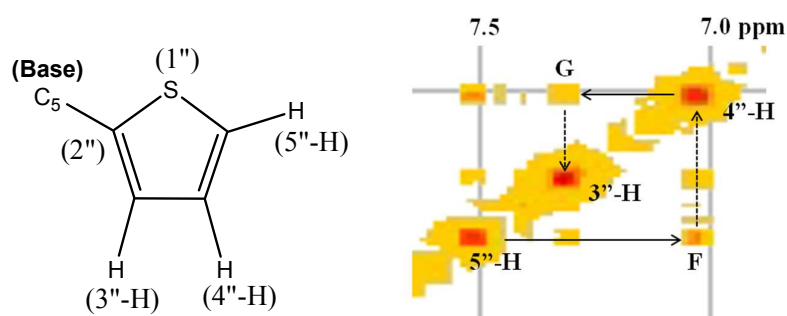


Figure 3, Left: chemical structure and labeling for the C₅-linked-thiophene moiety of **3b**; Right: a section of 2D ¹H-¹H NMR of **3b**. The cross peaks are labelled as **F** (4''-H and 5''-H) and **G** (3''-H and 4''-H).

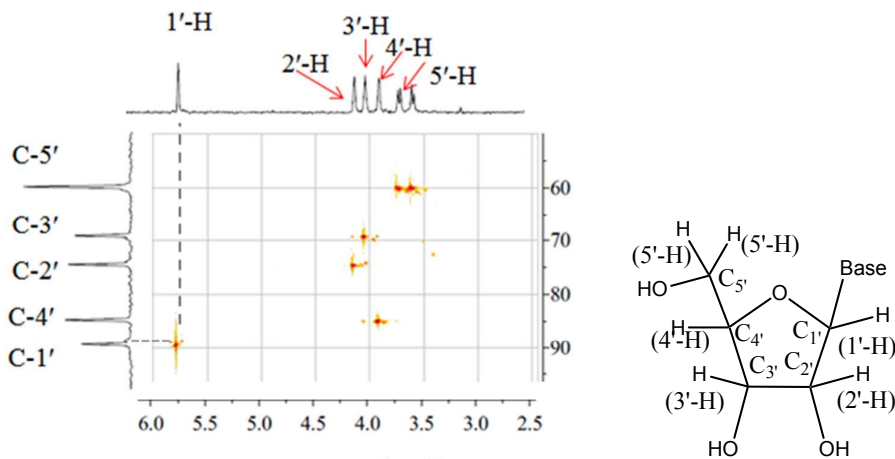


Figure 4 Left: Sugar section of ^1H - ^{13}C COSY spectrum of 4-thio-5-thiophene-ribose (3b). Right: Chemical structure and labelling of the sugar.

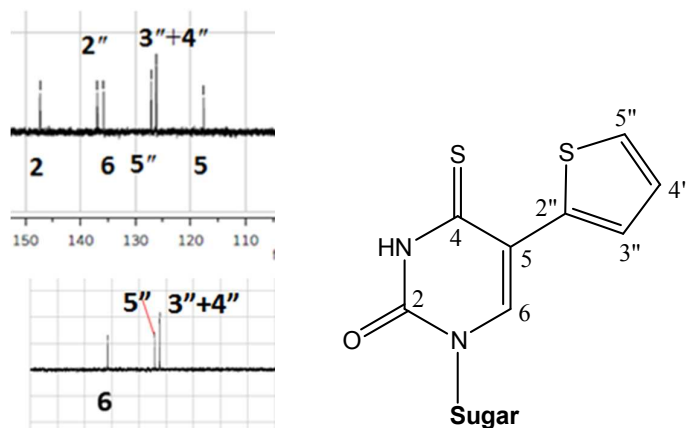


Figure 5. The signals of C-2 and C-5 (the base) and that of C-2'' (the ring) disappeared in the DEPT-135 and the signal of C-4 ($\delta=187$ ppm) is out of the scale. Left: Section of 1D ^{13}C NMR of **3b** (Upper) and its DEPT-135 (Lower); Right: Chemical structure and labelling of the base and 5-attached thiophene.

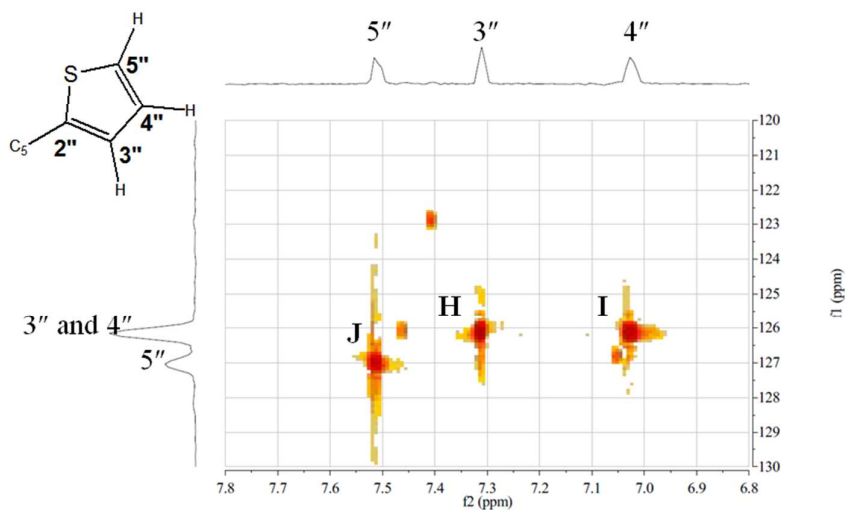


Figure 6. NMR signals of thiophene ring in ¹H-¹³C COSY spectrum of 4-thio-5-thiophene-ribouridine (**3b**).

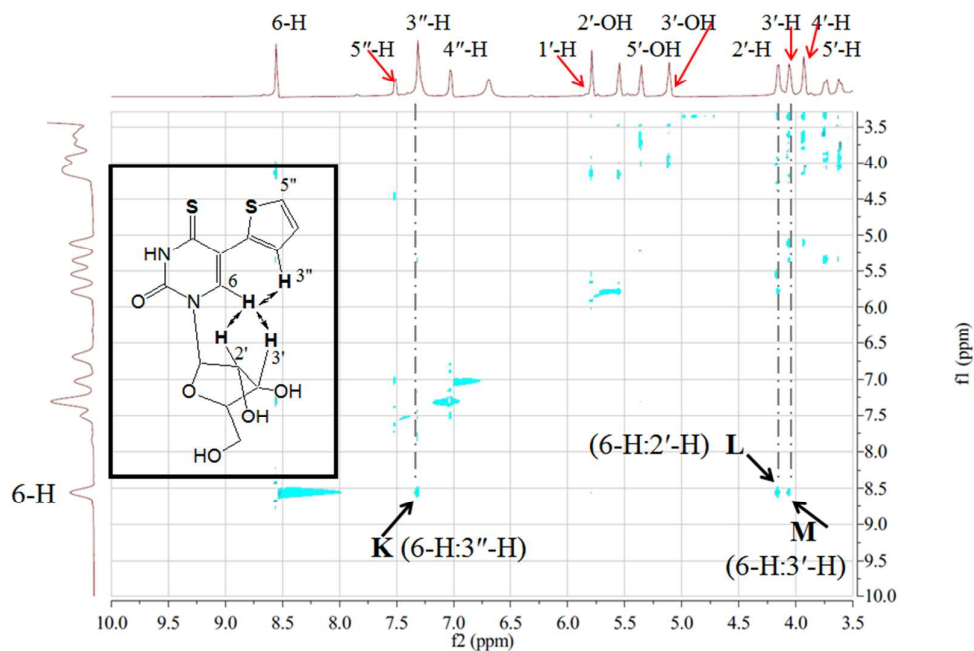


Figure 7. NOESY spectrum of 4-thio-5-(thiophene)-ribouridine (**3b**). Cross peak **K**: between 6-H of the base and 3''-H of the ring, cross peak **L**: between 6-H of the base and 2'-H of the sugar, cross peak **M**: between 6-H of the base and 3'-H of the sugar.

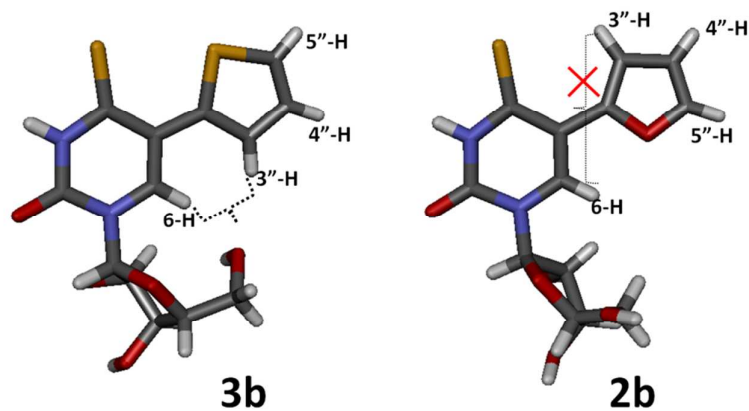


Figure 8: Molecular structures and absolute configurations of 4-thio-5-(thiophene)-uridine (**3b**) and 4-thio-5-(furan)-uridine (**2b**) determined by x-ray crystallography. Full structural data are deposited at the Cambridge Crystallographic Data Centre, depository number: 1479957 and 1479956.