Synthesis and Complete ^1H and ^{13}C Assignments of Thiazolo[3,2-*a*]quinolinium Derivatives

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Several hitherto unknown thiazolo[3,2-*a*]quinolinium salts derivatives were prepared and characterized. Two-dimensional high field nmr methods were employed to make complete assignments of the proton and carbon nmr spectra for these compounds.

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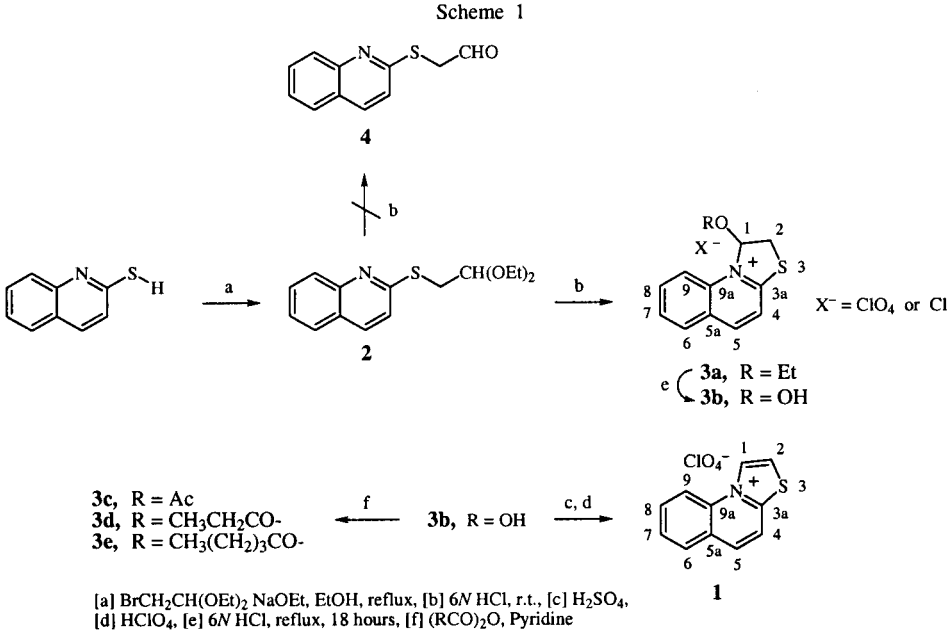
Introduction.

In a previous paper from this laboratory, we reported a two-dimensional nmr analysis of selected benzazolo-[3,2-*a*]quinolinium salts [1]. These salts display interesting biological properties including cytotoxicity against several tumoral cell lines [2], interaction with DNA by the intercalation mechanism [3], inhibition of DNA topoisomerases I or II [4] and induction to differentiation of HL-60 cells [5]. As part of our continued interest in this family of heterocycles, [1-2,6,7] we had to prepare thiazolo[3,2-*a*]quinolinium perchlorate **1**. This molecule was first synthesized by Bradsher and Lohr [8] by the sequence of reactions described in Scheme 1. The sequence commences with the reaction of 2-quinolinethiol with 2-bromoacetaldehyde diethyl acetal and sodium ethoxide to produce α-(2-quinolylthio)-acetaldehyde diethyl acetal **2**, followed by its acid hydrolysis with dilute hydrochloric acid. The product from this reaction (presumably the aldehyde) was treated with concentrated sulfuric acid to effect cyclization and dehydration. The final product, **1,** was isolated as the perchlorate salt. This work reports the isolation and characterization of several hitherto unknown intermediate products from this sequence of reactions and the assignment of their proton and carbon 13 chemical shifts by 2D nmr techniques.

Results and Discussion.

The synthetic sequence used was essentially that described, except that contrary to the previous report [8], all the intermediates from this reaction (see Scheme 1) were isolated, purified and identified (by combustion analysis and spectral data). The α-(2-quinolylthio)acetaldehyde diethyl acetal **2** was isolated as a pale yellow solid, mp 43-44°. Hydrolysis of **2** with 6*N* hydrochloric acid for 3 hours produced 1-ethoxy-1,2-dihydrothiazolo[3,2-*a*]quinolinium chloride **3a**, that was isolated and characterized as the perchlorate salt. When the hydrolysis time was extended to 18 hours under reflux, 1-hydroxy-1,2-dihydrothiazolo[3,2-*a*]-quinolinium chloride **3b** was obtained and also characterized as the perchlorate salt. Compound **3b** was converted to

Scheme 1



[a] $BrCH_2CH(OEt)_2$ NaOEt, EtOH, reflux, [b] 6*N* HCl, r.t., [c] H_2SO_4, [d] $HClO_4$, [e] 6*N* HCl, reflux, 18 hours, [f] $(RCO)_2O$, Pyridine

the corresponding ethanoate **3c,** propanoate **3d** and butanoate **3e** esters, respectively as described [9]. The crystal structure of ethanoate **3c** has been published elsewhere [9]. Treatment of **3b** with concentrated sulfuric acid as described [8] produced **1**.

The mechanistic details of this reaction were not explored. However, one important conclusion from this work is that the hydrolysis of α-(quinolylthio)acetaldehyde diethyl acetal, **2,** occurs in a stepwise fashion *via* the intermediacy of **3a** and **3b** as stable products. Furthermore **3a** is converted to **3b** in refluxing 6*N* hydrochloric acid, but this reaction is slow at room temperature. Interestingly, contrary to the previous report [8], there is no evidence for the intermediacy of aldehyde **4** in this reaction.

and the on the fully aromatic system **1**. Commencing with the proton spectrum, compounds **3a-e** display an ABX pattern in the aliphatic region corresponding to H-1, H-2a and H-2b. Protons H-2a and H-2b are diastereotopic, and therefore nonequivalent, thus producing an AB quartet (J = 12.9 Hz). This AB quartet is further split by interaction of H-2a and H-2b with H-1. The dihedral angles $\phi_{H\text{-}2a,H\text{-}1}$ and $\phi_{H\text{-}2b,H\text{-}1}$ for **3c**, obtained from X-ray crystallographic data, [9] are 30.2(1)° and 91.1(1)°, respectively. Therefore the observed coupling constants ($J_{H\text{-}2a,H\text{-}1} \approx 6$ Hz and $J_{H\text{-}2b,H\text{-}1} \approx 0$ Hz) are in agreement with the values calculated using the Karplus equation [10]. The corresponding dihedral angles for **3a, 3b, 3d-e** were calculated by semiempirical methods [11] and are in agreement with those obtained from X-ray data. Thus in **3b,** H-2a appears as

Table 1

^{13}C NMR Chemical Shifts δ in ppm of Thiazolo[3,2-*a*]quinolinium Salts Derivatives **1, 3a-e** [a]

Compound	C1	C2	C3a	C4	C5	C5a	C6	C7	C8	C9	C9a	CO	CH_2	CH_3
1	129.0	124.0	156.0	118.3	136.2	125.4	130.0	129.1	132.8	117.9	134.3	---	----	---
3a	94.7	34.1	166.0	118.6	146.1	126.3	130.3	128.4	135.1	118.1	136.9	---	63.6	14.8
3b	91.6	37.0	164.6	118.4	145.3	126.3	129.9	128.1	134.6	118.4	136.7	---	----	---
3c	87.7	35.8	168.0	118.8	146.0	126.2	130.7	128.6	135.6	117.4	136.3	168.5	---	20.4
3d	87.8	35.8	168.0	118.9	146.9	126.2	130.7	128.6	135.6	117.4	136.3	171.8	26.5	8.3
3e	87.7	36.0	168.1	118.9	147.0	126.2	130.8	128.7	135.7	117.4	136.3	171.0[b]	17.4	13.1

[a] In dimethyl-d_6 sulfoxide using tetramethylsilane as internal standard, δ in ppm. [b] -CH_2CO- , δ = 34.98 ppm.

Table 2

^{1}H NMR Chemical Shifts of Thiazolo[3,2-*a*]quinolinium Salts Derivatives **1, 3a-e** [a]

Compound	H1	H2a	H2b	H4	H5	H6	H7	H8	H9
1	9.79 (d, 4.1)	8.67 (d, 4.1) [b]	---	8.72 (d, 9.3)	8.77 (d, 9.3)	8.42 (d, 8.0)	7.99 [c]	8.18 [c]	8.96 (d, 8.6)
3a [d]	7.41 (d, 6.3)	4.24 (dd, 13.4, 6.3)	4.09 (d, 13.4)	8.26 (d, 8.9)	8.99 (d, 8.0)	8.35 (d, 8.1)	7.92 [c]	8.20 [c]	8.31 (d, 8.9)
3b	7.42 (d, 6.6)	4.19 (dd, 12.9, 6.6)	3.75 (d, 12.9)	8.16 (d, 9.0)	8.89 (d, 9.0)	8.28 (d, 8.4)	7.88 [c]	8.14 [c]	8.29 [e]
3c [f]	[g]	4.33 (dd, 13.8, 6.3)	4.16 (d, 13.8)	8.31 (d, 8.3)	9.04 (d, 9.0)	8.36 (d, 8.3)	7.88 [c]	[g]	8.06 (d, 8.0)
3d [g]	8.21 (d, 6.3)	4.35 (dd, 13.8, 6.3)	4.18 (d, 13.8)	8.33 (d, 9.0)	9.03 (d, 9.0)	8.38 (d, 8.1)	7.94 [c]	8.15 [c]	8.07 (d, 8.0)
3e [h]	8.21 (d, 6.0)	4.24 (dd, 13.8, 6.0)	4.13 (d, 13.8)	8.30 (d, 9.0)	9.04 (d, 9.0)	8.36 (d, 9.0)	7.91 [c]	8.18 [c]	8.03 (d, 8.7)

[a] In dimethyl-d_6 sulfoxide using tetramethylsilane as internal standard, δ in ppm (multiplet), (J Hz)). [b] Olefinic proton H2. [c] Apparent triplet. [d] CH_3- 1.14 (t, 7.0), CH_2- 3.95 (q, 7.0). [e] Coincides with H6. [f] CH_3- 2.10 (s). [g] The unresolved multiplet at 8.19-8.42 ppm corresponds to H1 and H8. [h] CH_3- 1.04 (t, 7.5), CH_2- 2.42 (q, 7.5). [i] CH_3- 0.811 (t, 7.4), CH_2- 1.51 (m, 7.4), CH_2CO 2.34 (td, 7.4, 2.3).

Assignment of the ^{1}H and ^{13}C nmr spectra of compounds **1, 3a-e** (Tables 1 and 2) is based on nOe difference experiments, INAPT, and several two-dimensional nmr techniques (COSY, HETCOR and HMBC). The proton and carbon spectra for **1, 3a-d** were recorded in dimethyl-d_6 sulfoxide at observation frequencies of 300.15 and 75.48 MHz, respectively. The proton and carbon spectrum of **3e** was recorded in dimethyl-d_6 sulfoxide at observation frequencies of 500.13 and 125.77 MHz, respectively. Our discussion will be concerned only with the proton and carbon nmr spectrum of the thiazolo[3,2-*a*]quinolinium ion moiety in **1, 3a-e**. Assignment of the nmr signals for the appendixes is straightforward. First we will focus on **3a-e** doublet of doublets (at δ = 4.19 ppm, J = 12.9 and 6.6 Hz) and H-2b as a doublet (at δ = 3.75 ppm, J = 12.9 Hz). For **3b**, the down field doublet at 7.42 ppm (J = 6.6 Hz) corresponds to H-1 as confirmed by the COSY spectrum. For esters **3c-e,** H-1 is shifted down field (*i.e.* δ = 8.21 ppm, d, J = 6.3 Hz for **3e**).

The aromatic region, in the 300 MHz proton nmr spectra, of the thiazolo[3,2-*a*]quinolinium ion moiety in **3a-c** show a downfield signal (δ = 8.99-9.03 ppm, J = 9.0 Hz, 1H), unresolved multiplets between δ = 8.28-8.36 ppm and a pseudo triplet between δ = 7.88-7.91 ppm (1H). Since **3c** shows greater resolution than **3b**, we decided to synthesize two additional ester derivatives, **3d** and **3e**, incorporating

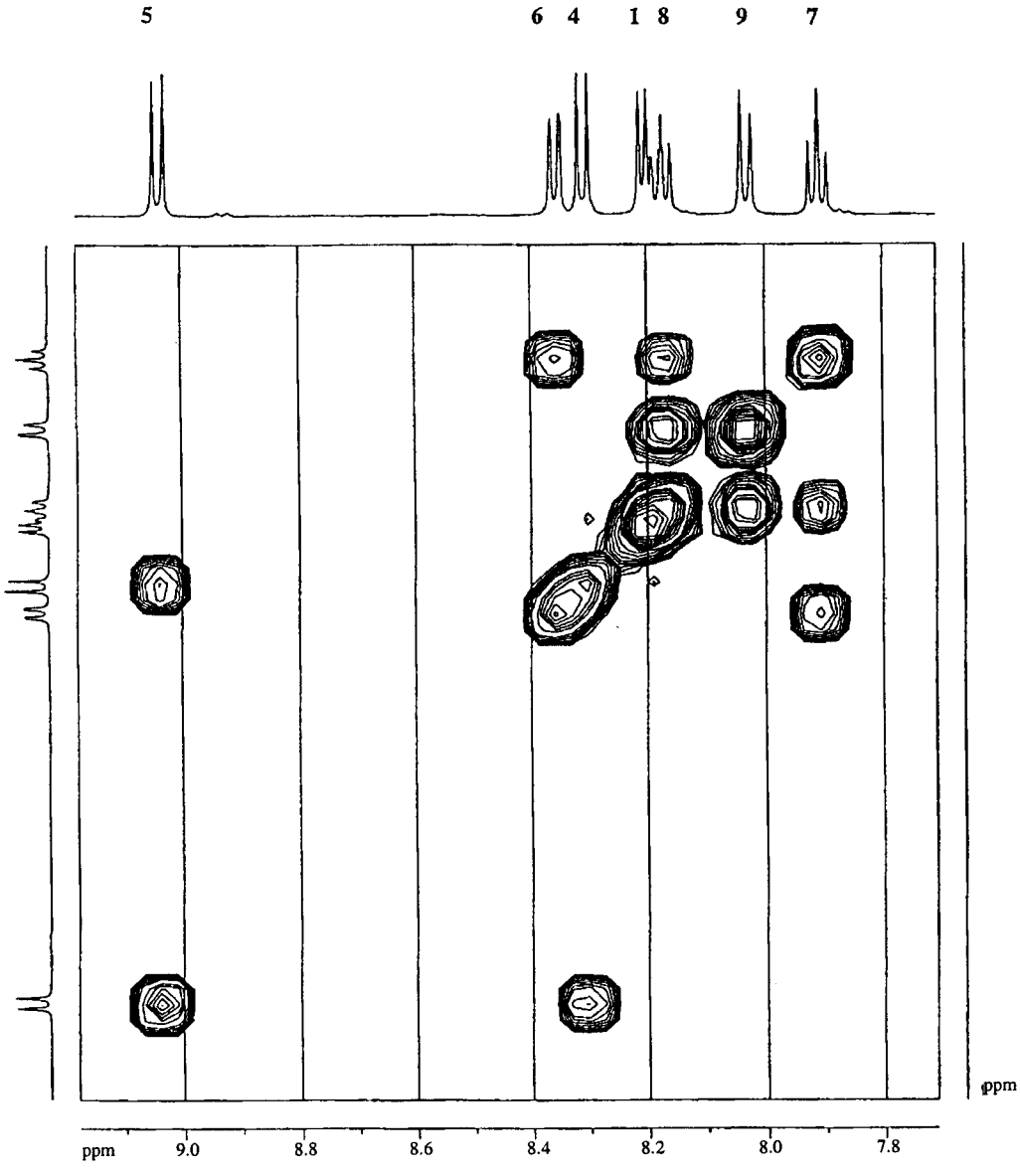


Figure 1. Contour plot of the 500 MHz 2D ^1H COSY spectrum of the aromatic region of 1-butyroxy-1,2-dihydrothiazolo[3,2-*a*]quinolinium perchlorate (**3e**).

longer aliphatic chains. As expected the 300 MHz proton nmr spectra of **3d** and **3e** show greater resolution in the aromatic region than **3a-c**. The complete and unequivocal assignment of the aromatic of the ^1H spectrum of these molecules was possible because the 500 MHz proton nmr spectrum of **3e** is well resolved (see Figures 1 and 2).

Two alternative approaches are available for the assignment of the proton signals in **3a-e**. These approaches will fucus on either the resolved downfield doublet or the resolved upfield pseudo triplet. On the basis of chemical intuition one may be tempted to assign the downfield doublet to H-9 and the high field pseudo triplet to H-7. Analysis of the 500 MHz COSY spectrum of **3e** (Figure 1) shows that these intuitive assignments are correct for H-7, but not for H-9. The arguments are as follows: The low field signal at δ = 9.04 (d, J = 9.0, 1H) and that at δ = 8.30 ppm (d, J = 9.0 Hz, 1H) are mutually correlated, and neither show additional correlations. In order to obtain additional information about the identity of the low field doublet, nOe difference experiments [16] were performed on **3e**. Thus irradiation of the low field doublet produces a 52% nOe enhancement for the signals between δ = 8.40-8.25 ppm (H-4 and H-6), while the remaining signals are not affected. Therefore the low field doublet is assigned to H-5 (δ 9.04, J = 9.0 Hz, 1H), the doublet at δ = 8.30 ppm (d, J = 9.0 Hz, 1H) to H-4 and the signal at δ = 8.36 ppm

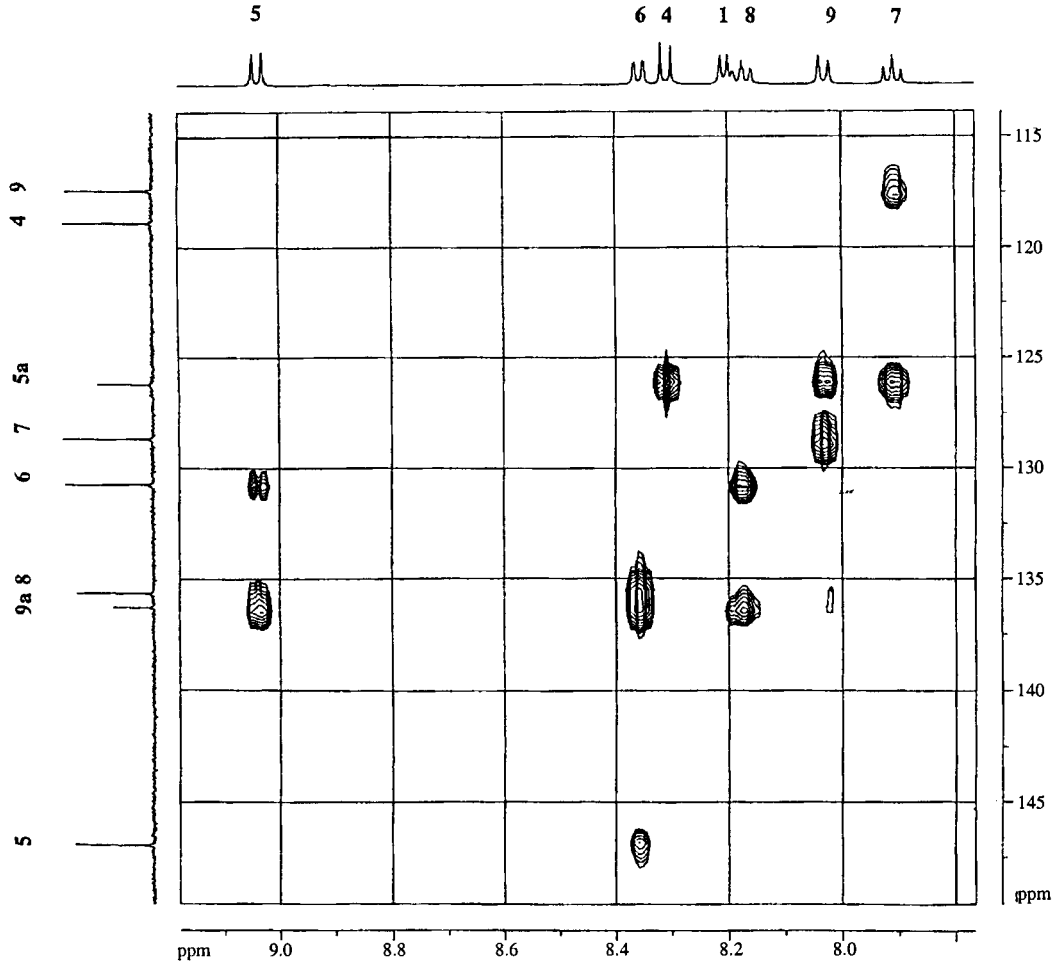


Figure 2. Contour plot of the 500 MHz 2D HMBC spectrum of 1-butyroxy-1,2-dihydrothiazolo[3,2-*a*]quinolinium perchlorate (**3e**).

(d, J = 9.0 Hz, 1H) to H-6. With H-4, H-5 and H-6 assigned, the assignment of H-7, H-8 and H-9 is straightforward. Thus the pseudo triplet at δ = 7.91 ppm is correlated with the signal at δ = 8.36 ppm (d, J = 9.0 Hz, 1H), already assigned to H-6, and with the pseudo triplet at δ = 8.18 assigned as H-8. Finally H-8 is correlated with the signal at δ = 8.03 (d, J = 8.7 Hz, 1H) assigned to H-9. The signal at δ = 7.21 ppm (d, J = 6.0 Hz, 1H) was already assigned to H-1. These assignments are in agreement with the HMBC data for **3e** (see Figure 1).

With the ^{1}H nmr resonances of **3a-e** assigned, we proceeded to assign those in the unsaturated heterocyclic ring system **1**. As concerns **1**, two signals are seen with triplet appearance (centered at δ = 7.99 and 8.18 ppm) corresponding to H-7 and H-8, respectively. The COSY spectrum of **1** shows homonuclear coupling of H-8 with the doublet signal at 8.96 ppm (J = 8.6 Hz). Therefore this signal is assigned to H-9. In turn H-7 shows correlation with the signal at δ = 8.42 (J = 8.0 Hz), which is assigned to H-6. The AB pattern observed in the spectrum of **1**, with resonances centered at 8.72 and 8.77 ppm (J_{AB} = 9.3 Hz) corresponds to H-4 and H-5, respectively. Since **1** is a fully aromatic system, H-9 (8.96 ppm) is a bay proton and as expected is shifted downfield with respect to H-5 (8.77 ppm). This situation contrasts with that in compounds **3a-e**, where H-9 is adjacent to the saturated thiazolo portion of the molecule and the system is not fully aromatic. Therefore in **3a-e**, H-9 is shifted upfield with respect to H-5. The protons attached to the double bond in the thiazole ring of **1**, H-1 and H-2 are readily identified by their small coupling constant and COSY data, as doublets centered at 9.79 and 8.67 ppm (J = 4.1 Hz), respectively.

Once the proton resonances in **3a-e** had been established, aliphatic carbons can be dealt with straightforwardly through HETCOR experiments (Table 1). Thus for **3b**, C-1 and C-2 resonate at 91.6 and 37.0 ppm, respectively. The peak corresponding to C-1 appears as a broad singlet for all the compounds studied due to the scalar relaxation caused by the quadrupolar effect of the nitrogen atom. Assignments of the quaternary carbons C-3a, C-5a and C-9a was made on the basis of spin-lattice relaxation times of compound **1** as measured by the inversion-recovery method [12]. Carbon C-5a was expected, if a dipole-dipole mechanism of relaxation

was dominant, to relax more efficiently than C-9a, since it is flanked by H-5 and H-6, whereas, C-9a is adjacent to only one proton. Quaternary carbon C-3a is readily assigned on the basis of its chemical shift as the lowest resonating carbon. In the 1,2-unsaturated derivative, **1**, it was found that the upfield resonance at 125.4 ppm has a T_1 of 14 s, whereas the down field resonance at 134.3 ppm has a T_1 of 17 s. Based on these results, C-5a resonates at higher field as compared to C-9a, and the α carbons are more deshielded than the β carbons. In the 1,2-unsaturated derivative, **1**, C-3a resonates at 156.0 ppm, C-9a resonates at 134.3 ppm, and C-5a at 125.4 ppm. The quaternary carbons of **3d** and **3e** were readily assigned on the basis of selective INAPT and HMBC experiments (**3e**, see Figure 2). The, 500 MHz, HMBC [15] experiment with **3e** was optimized for 166 Hz ($\Delta_1 = {}^1/_2J_{CH}$), in order to suppress one-bond responses and 10 Hz ($\Delta_2 = 1/{}^nJ_{CH}$) for long range responses. Thus irradiation of H-8 shows correlation with C-9a and C-6, irradiation of H-9 shows correlation with C-7 and C-5a, irradiation of H-6 shows correlation with C-9a , C-8 and C-5, irradiation of H-5 shows correlation with C-9a and C-3a and irradiation of H-4 shows correlation with C-9a and C-5a. Thus in **3e,** C3a, C5a and C9a resonate at δ = 168.1, 147.0 and 117.4 ppm, respectively. The complete nmr data of these compounds are reported in Tables 1 and 2.

EXPERIMENTAL

All starting materials were commercially available and used as received, except where indicated. The ^1H and ^{13}C nmr spectra were recorded on a General Electric QE-300 (using a 5 mm C/H dual probe) operating at a frequency of 300.15 and 75.48 MHz for ^1H and ^{13}C, respectively and equipped with a Nicolet 1280 data system and a 293-C pulse programmer or on a Bruker DRX500 (using a 5 mm broadband probe) operating at a frequency of 500.13 MHz and 125.77 for ^1H and ^{13}C, respectively. The quantity of sample ranged from 30 to 150 mg, all dissolved in 0.5ml of dimethyl-d_6 sulfoxide. The chemical shifts were referenced relative to tetramethylsilane as internal standard. The conditions of measurement of the ^1H and ^{13}C spectra were as follows: pulse width, 8.75 μs (90°) and 9.60 μs (90°), respectively; spectral width 3311 Hz and 14705 Hz, respectively; data points 16 K and 32 K, respectively; repetition time, 3.47 s and 1.61 s.

The COSY spectra were recorded using the sequence described by Bax and Freeman [13]. The spectral widths were 3311 Hz in both dimensions (F_1, F_2). The spectra were collected as 256 x 1K block of data, and was processed to a final 512 x 512 real points matrix (zerofilling the F_1 dimension) using sinebell multiplications in each dimension followed by symmetrization of the final data matrix.

The heteronuclear chemical shift correlation (HETCOR) spectra were obtained using the pulse sequence described by Bax and Sarkar [14]. The spectra were obtained from a 128 x 2K data matrix with 128 accumulations performed for each t_1; the delay times used were 1.85 ms ($^1/_4J_{CH} = \Delta_1$) and 2.5 ms (1/3 $J_{CH} = \Delta_2$), optimizing for a coupling constant of approximately 135 Hz. Heteronuclear multiple bond correlations (HMBC) were obtained as described by Bax and Summers [15]. The spectra were obtained from a 256 x 4 K data matrix with 96 accumulations performed for each t_1. The delay times were 3.0 ms (1/2 $J_{CH} = \Delta_1$), to suppress one-bond responses, and 50 ms ($1/{}^nJ_{CH} = \Delta_2$) for evolution of long range couplings (10 Hz). The nOe difference spectral conditions using a single-frequency ^1H probe were as follows [16]: pulse width, 3.0 μs (90°), acquisition time, 5.16 s, pre-acquisition delay, 5.0 s, number of scans, 96. Saturation of the appropriate resonance was applied during the pre-acquisition delay on alternate scans, and phase cycling of the pulse used to effect alternate addition and subtraction of the free induction decays.

The quaternary carbons were assigned using the selective INEPT (INAPT) pulse sequence described by Bax [17], where the soft pulse was set to obtain a value of $\gamma H_2 = 21.3$ Hz, therefore giving a soft 90° pulse with of 11.7 ms. The spectral widths were 14705 Hz, and 1024 transients were accumulated; per experiment and Δ_1 and Δ_2, respectively, or both were set to 50 ms and 36 ms. The T_1 values were calculated by the inversion-recovery method [12].

α-(2-Quinolylthio)acetaldehyde Diethyl Acetal (**2**).

Compound **2** was prepared as described previously [8] and isolated as a pale yellow solid, mp 43-44°; ^1H nmr (dimethyl-d_6 sulfoxide, 300 MHz): δ 7.99-7.21 (m, 6H, aromatic), 4.89 (t, 1H, -CH), 3.83 (m, 6H), 1.29 (t, 6H, 2 -CH_3); ^{13}C nmr (dimethyl-d_6 sulfoxide, 75.48 MHz): δ 158.2, 147.5, 135.1, 129.3, 127.4, 127.1, 125.5, 124.8, 120.3, 101.9, 61.55, 32.2, 14.9.

Anal. Calcd. for $C_{15}H_{19}NO_2S$: C, 64.98; H, 6.85; N, 5.05. Found: C, 65.00; H, 6.89; N, 5.04.

Thiazolo[3,2-*a*]quinolinium Perchlorate (**1**).

This compound was prepared as described previously [8] in 84% yield. Recrystallization from water gave pure **1** as white crystals: mp 181-182.5° (lit [8] 181-183°).

1-Ethoxy-1,2-dihydrothiazolo[3,2-*a*]quinolinium Perchlorate (**3a**).

A solution of 3.60 g (10.0 mmoles) of **2** in 50 ml of 6*N* hydrochloric acid was allowed to stand at room temperature for 6 hours. The solution was concentrated under vacuum and poured into 200 ml of ether and placed in the refrigerator for 3 hours. The ether was decanted and 50 ml of water was added, followed by 35% percent perchloric acid to precipitate the desired product as a light yellow solid. Recrystallization from water gave pure **3b** (60%), mp 165-166°.

Anal. Calcd. for $C_{13}H_{14}ClNO_5S$: C, 47.04; H, 4.22; N, 4.22. Found: C, 46.90; H, 4.17; N, 4.17.

1-Hydroxy-1,2-dihydrothiazolo[3,2-*a*]quinolinium Perchlorate (**3b**).

A solution of 1-ethoxy-1,2-dihydrothiazolo[3,2-*a*]quinolinium perchlorate (**3a**) (140 mg, 0.400 mmole) in 25 ml of 6*N* hydrochloric acid was refluxed for 18 hours. The solvent was evaporated under vacuum and the residue recrystallized from water to give pure **3b** as a pale yellow solid, mp 69-70°.

Anal. Calcd. for $C_{11}H_{10}ClNO_5S$: C, 43.50; H, 3.32; N, 4.61. Found: C, 43.33; H, 3.33; N, 4.54.

General Procedure for the Synthesis of Esters **3c-e** [9].

To a solution of **3b** (150 mg, 0.500 mmole) in 1 ml of the corresponding anhydride, was added 3 drops of dry pyridine and the resulting solution was stirred a room temperature for 30 minutes. The product was isolated by vacuum filtration and recrystallyzed from ethanol.

1-Acetoxy-1,2-dihydrothiazolo[3,2-*a*]quinolinium Perchlorate (**3c**).

Following the general procedure, this compound was prepared in a quantitative yield from **3b** and acetic anhydride. Recrystallization from ethanol gave **3c** as a white solid, mp 230-232°.

Anal. Calcd. for $C_{13}H_{12}ClNO_6S$: C, 45.15; H, 3.47; N, 4.05. Found: C, 45.23; H, 3.51; N, 4.02.

1-Propionoxy-1,2-dihydrothiazolo[3,2-*a*]quinolinium Perchlorate (**3d**).

Following the general procedure, this compound was obtained in quantitative yield from **3b** and propionic anhydride. Recrystallization from ethanol produced pure **3d** as a pale yellow solid, mp 175-176°.

Anal. Calcd. for $C_{14}H_{14}ClNO_6S$: C, 46.99; H, 3.87; N, 3.92. Found: C, 46.91; H, 3.93; N, 3.87.

1-Butyroxy-1,2-dihydrothiazolo[3,2-*a*]quinolinium Perchlorate (**3e**).

Following the general procedure, this compound was prepared in quantitative yield from **3b** and butanoic anhydride. Recrystallization from ethanol produced **3e** as pale yellow crystals: mp 163-165°.

Anal. Calcd. for $C_{15}H_{16}ClNO_6S$: C, 48.45; H, 4.31; N, 3.77. Found: C, 48.30; H, 4.36; N, 3.80.

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