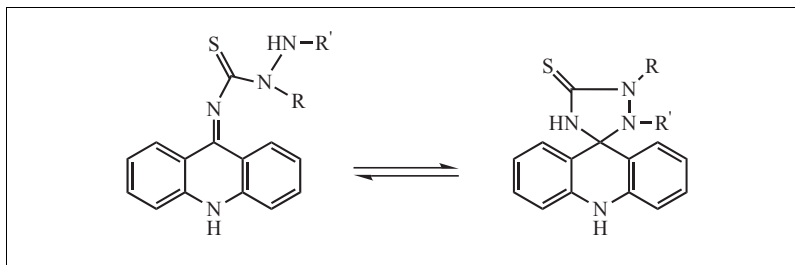


Karel D. Klika,^{*,**a} Eva Balentová,^{a,b} Juraj Bernát,^b Ján Imrich,^b Martina Vavrušová,^b
Erich Kleinpeter,^c Kalevi Pihlaja^a and Andreas Koch^c

^a Department of Chemistry, University of Turku,
Vatselankatu 2, FIN-20014 Turku, Finland

^b Department of Organic Chemistry, P. J. Šafárik University,
Moyzesova 11, SK-04167 Košice, The Slovak Republic

^c Department of Chemistry, University of Potsdam,
Am Neuen Palais 10, D-14415 Potsdam, Germany
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The regioselectivities of methyl- and phenylhydrazine with acridin-9-yl isothiocyanate (thus yielding thiosemicarbazides with acridine substituted on the urea-type side) were examined. Methylhydrazine regioselectivity was high with the α -nitrogen atom overwhelmingly more nucleophilic than the β -nitrogen atom; phenylhydrazine regioselectivity was poor but varied with the solvent and only in the case of ethanol was nucleophilic predominance of the α -nitrogen atom pronounced. Of note, whilst both phenyl thiosemicarbazides were present in solution only as spiro forms, the methyl product was present as an equilibrium mixture of open-chain and spiro thiosemicarbazides. Reactions on the NH_2 blocked analogue of methyl acridin-9-ylthiosemicarbazide (1-isopropylidene-2-methylthiosemicarbazide) were also examined. Interestingly, present in the starting material itself was a structural motif of novelty wherein a triazolethione represented the major species of an equilibrium between cyclic and open-chain forms.

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Introduction.

Acridines have long been appreciated as antimalarial and bactericidal compounds and, in recent times, as antitumor agents [1–3]. Special attention has been paid to derivatives of 9-aminoacridines as potent inhibitors of enzymes topomerase I and II responsible for regulation of topology, replication, and transcription of DNA (*e.g.* anti-leukemic drug amsacrine [4]). Other aminoacridines have served as vectors increasing the specificity and cytotoxicity of modified DNA molecules used in chemotherapy [5]. Another well-known application is the anticholinesterase drug 9-amino-1,2,3,4-tetrahydroacridine (TacrinTM) [2] for the treatment of Alzheimer's disease. Promising pharmaceutical effects, moreover, could be expected from acridine intercalators composed of a connecting aromatic or heterocyclic moiety. Thus, this study and the two succeeding [6,7] ones were inspired by our interest in the preparation of heterocyclic structures from suitably substituted precursors which, in combination with acridines, can afford unusual structures [7–9]. But as the

complexity of the system builds up, the potential for esoteric structures rises rapidly but oft times it is the propensity of acridinyl systems to form spiro compounds that comes to the fore.

In this work, as part of an examination of thiosemicarbazides containing the acridine moiety (an extension of our long-standing studies with acridine-[8,10–15] and anthracene-substituted [16,17] thioureas for the purposes of obtaining new and novel type structures concomitant with potential biological applications), we focused on acridines substituted at the urea-type nitrogen atom {Acr-NH-C(=S)-NR-NH₂, prepared from acridin-9-yl isothiocyanate and methyl/phenylhydrazine}. In the following article [6], thiosemicarbazides with acridine on the carbazide side {Acr-NH-NH-C(=S)-NH-R, prepared from acridin-9-ylhydrazine and isothiocyanates} were examined. This seemingly minor distinction of acridine substitution profoundly changes the susceptibility of C-9 to nucleophilic attack, as well as many other reaction routes, thus leading to divergent pathways

regarding reaction, structure, conformation, configuration, and the effect on the chemical shift of H-1 in/of the resultant products. One of the underlying themes in acridine-based work seems to be the pernicious desire of the N-10 nitrogen atom to capture a proton [9,18] and thus drive the formation of spiro or iminyl structures for the acridine moiety (*i.e.* form 9,10-dihydro structures). Thus, in the former case, rendering C-9 susceptible to nucleophilic attack. For example, in the accompanying study [6], it was noted that for the majority of the products a coplanar acridin-9-yl hydrazone structure was present with extended conjugation based on the propensity of the N-10 acridine nitrogen to deprive N-11 of its proton and which results in the formation of a 9,10-dihydroacridin-9-ylidene structure attached through a C₉=N₁₁ double bond to the thiosemicarbazide moiety. Such extended coplanarity is of interest in light of the fact that the biological activity of acridines is generally ascribed to their intercalation into the stacked base pairs of the DNA precisely due to their planarity. Due to the greater susceptibility of C-9 to nucleophilic attack and hence formation of spiro structures, and to steric hindrance in cases when this did not happen due to closer proximity to the thiocarbonyl bond, extended planarity as such was not a prominent feature of the compounds realized here. Overviews of the syntheses of thiosemicarbazides have been described [19,20] and aliphatic thiosemicarbazides in particular, have been systematically studied [21].

Results and Discussion.

Structural determinations and signal assignments were accomplished by the application of the usual combination of ¹H, ¹³C, and ¹⁵N chemical shifts assisted by DEPT, NOE difference, selective INEPT, and ¹⁵N INEPT 1-D experiments and DQF COSY, NOESY, ¹³C-¹H}-CHSHFT, ¹H-¹³C} and ¹H-¹⁵N}-HSQC, ¹H-¹³C}- and ¹H-¹⁵N}-HMBC 2-D experiments. Experiments were performed with standard, vendor-supplied pulse sequences except in the case of the sign determination of long-range couplings [22] used for distinguishing between couplings over 2- or 3-bonds. The ¹H, ¹³C, and ¹⁵N chemical shifts of the compounds are compiled in Tables 1 and 2 whilst the homonuclear couplings extracted by spin simulation [23] are presented only for selected cases given the close similarity of the couplings in the acridine moiety (see also ref [6]).

To limit repetitious descriptions, structural determinations are not described in detail and structures are essentially just presented—but based on thorough analysis—with only pivotal points pertaining to unexpected or peculiar structures being described explicitly. For example, S-methylation is readily indicated by the chemical shift of the methyl carbon which is strongly

shielded (13–15 ppm). The equivalence of the off-central axis nuclei of the acridine moiety (the outer rings) is indicative of either an acridine moiety (*i.e.* not 9,10-dihydro) or spirocyclization (excepting the presence of an asymmetric center), but the distinction between these two is trivial based on the carbon chemical shifts, most notably C-9 which lies in the range 60–80 ppm for the latter structure. The non-equivalence of the outer rings of acridine therefore indicates either the presence of an imidyl C₉=N₁₁ double bond or an asymmetric carbon if spiro formation has occurred. Generally, for the case of an imidyl C₉=N₁₁ bond, inversion at N-11 was slow enough to render the off-central axis nuclei of the acridine moiety non-equivalent.

As noted above, a recurring theme even in this work is the retention by N-10 of a labile H. This is surprising as the conjugation between the C₉=N₁₁ and C₁₂=S double bonds should be limited given the likely steric hindrance for both *s-cis* and *s-trans* configurations. The likely twist therefore, about the N₁₁-C₁₂ bond, to relieve this hindrance has a discernable effect on H-1: a pronounced shielding with respect to H-8 was generally observed. Residence of H-1 in the shielding cone of the C₁₂=S double bond due to such a twist is held to account for this observation. This is in contrast to the strong deshielding of H-1 observed in the instances when the conjugated system remains planar [6]. However, though N-10 clearly has a propensity to attain H, leading to 9,10-dihydro structures with C-9 either as an imino or a C_q spiro carbon, the likely preference was never evident, nor even the attainment of a 9,10-dihydro structure was assured over other structures. The unambiguous positioning of a labile H at N-10 was usually provided by strong NOEs to H-4 and H-5, as well as HMBC correlations to C-8a and C-9a and even to C-4 and C-5 on occasion. Even the chemical shift of a proton when located on N-10 was suggestive, with a relatively sharp signal generally in the range of 10.5–11.7 ppm for a hydrazone species (C₉=N₁₁) or 9.2–9.8 ppm for a C-9 spiro structure.

The reaction of acridin-9-yl isothiocyanate (**1**) with methylhydrazine was found to be regiospecific whereby the more nucleophilic α nitrogen of methylhydrazine attacked the isothiocyanate carbon to preferentially form the N-13 methylated thiosemicarbazide product {4-(9,10-dihydroacridin-9-ylidene)-2-methyl-thiosemicarbazide, **2**} without evidence for the presence of the N-14 methylated thiosemicarbazide. However, the identity of the reaction product and its regiospecificity was not immediately apparent as two sets of signals were observed by NMR (*ca.* 96:4) and furthermore, in the ¹H NMR spectrum of the major species **2**, three distinct one-proton NH signals were observed, suggestive of an N-14 methylated product rather than **2**. This prompted, for comparative purposes, the synthesis of the non-methylated analogue, *viz.* the

Table 1
¹³C and ¹⁵N NMR experimental chemical shifts^a (δ in ppm) for compounds **2–13** in DMSO-d₆ at 25 °C and calculated chemical shifts at the B3LYP/cc-pVTZ level of theory for all modeled structures.

	C-1	C-2	C-3	C-4	C-4a	C-5	C-6	C-7	C-8	C-8a	C-9	C-9a	N-10	C-10a	N-11	C-12	N-13	N-14	others
2 , exp	127.17	120.51	132.55	117.21	140.36	115.81	131.58	121.03	126.23	117.93	155.38	113.48	-266.7	138.15	-106.6	177.84	-234.2	-273.3	39.28 (NCH ₃)
2	135.5	122.3	134.9	115.5	142.4	115.0	134.4	123.8	132.8	125.3	150.0	119.0	-261.5	141.4	-83.4	199.6	-231.8	-284.3	39.2 (NCH ₃)
3 , exp	127.31	119.39	131.13	116.53	141.02	115.02	130.11	120.78	125.44	118.94	141.68	114.13	-	138.41	-	177.72	-	-	40.6 (NCH ₃)
4 , exp	126.92	119.69	129.06	113.97	138.04	113.97	129.06	119.69	126.92	119.10	74.22	119.10	-282.0	138.04	-245.7	177.40	-246.8	-243.1	34.40 (NCH ₃)
4	130.4	123.7	132.0	114.4	141.0	114.2	132.9	122.9	130.1	122.2	79.2	125.0	-268.1	143.4	-243.8	188.5	-236.6	-235.5	32.5 (NCH ₃)
5 , exp	135.3	123.0	132.0	113.2	140.3	114.1	132.8	123.4	131.2	126.5	76.7	125.1	-271.6	139.7	-232.1	185.0	-234.7	-213.8	35.3 (NCH ₃)
5	127.06	119.96	129.49	114.31	138.60	114.31	129.49	119.96	127.06	118.48	75.09	118.48	-281.7	138.60	-237.7	176.48	-	-243.5	122.14 (ortho), 124.50 (para), 127.93 (meta), 140.05 (ipso)
5 ^o	135.0	122.8	134.6	115.6	142.6	114.7	134.3	123.6	133.1	124.9	147.3	120.1	-262.1	141.9	-86.7	195.6	-214.0	-280.4	127.8 (ortho), 126.6 (para), 129.6 (meta), 148.7 (ipso)
5	135.8	122.8	135.5	115.8	142.5	115.5	134.9	123.9	132.5	125.2	151.9	118.7	-260.1	141.6	-79.2	197.7	-214.2	-288.9	129.7 (ortho), 127.1 (para), 129.2 (meta), 149.3 (ipso)
5	130.9	124.0	132.3	114.5	140.7	114.3	133.1	123.1	130.2	121.4	78.5	124.5	-268.3	143.4	-235.6	186.1	-215.7	-233.4	123.3 (ortho), 126.2 (para), 130.0 (meta), 143.7 (ipso)
6 , exp	128.41	119.55	129.19	113.93	137.85	113.93	129.19	119.55	128.41	118.20	82.16	118.20	-282.3	137.85	-270.8 ^b	174.81	-232.9	-	116.58 (ortho), 121.09 (para), 127.89 (meta), 144.77 (ipso)
6 ^o	135.7	122.6	135.7	115.7	142.4	114.6	135.1	124.1	134.1	125.0	154.9	118.1	-259.2	141.3	-90.2	197.0	-240.9	-269.8	113.7 (ortho), 121.4 (para), 131.8 (meta), 153.3 (ipso)
6	136.6	122.8	132.8	113.4	140.4	114.4	132.8	123.2	133.4	122.1	88.1	125.2	-271.6	140.3	-220.3	185.1	-226.1	-216.2	120.5 (ortho), 124.9 (para), 131.2 (meta), 150.9 (ipso)
7 , exp	128.32	119.59	131.75	116.59	140.67	115.58	130.79	120.85	125.79	118.83	151.37	114.49	-270.3	138.30	-78.3	160.20	-255.8	-301.6	34.87 (NCH ₃)
7	136.0	122.6	134.8	115.3	142.5	115.0	134.4	123.5	132.6	126.2	155.6	122.3	-262.1	141.7	-99.6	168.1	-254.8	-302.8	37.3 (NCH ₃)
7 ^s	136.2	123.0	135.0	115.3	142.4	115.0	134.4	123.5	132.3	126.1	155.7	122.2	-262.0	141.7	-97.0	168.3	-255.2	-301.2	34.2 (NCH ₃)
8	128.8	123.4	131.6	114.5	141.2	113.9	132.5	122.7	129.9	123.5	77.9	127.8	-268.0	143.2	-268.5	166.9	-262.2	-243.9	33.6 (NCH ₃)
8	127.45	120.82	132.29	116.65	139.55	116.65	132.29	120.82	127.45	116.40	147.14	116.40	-	139.55	-	183.43	-216.9	-49.6	19.51, 23.76 (2 × CH ₃), 30.58 (NCH ₃), 175.38 (NCq)
9	125.42	126.14	130.35	129.29	139.98	129.29	130.35	126.14	125.42	125.47	149.44	125.47	-69.5	139.98	-245.7	179.00	-242.6	-257.3	24.23 (2 × CH ₃), 35.46 (NCH ₃), 81.78 (NCq)
10	125.89	124.28	135.29	118.66	139.67	118.66	135.29	124.28	125.89	115.53	156.96	115.53	-244.3	139.67	-147.0	165.87	-219.7	-57.0	14.65 (SCH ₃), 20.27 (c CH ₃), 24.17 (tr CH ₃), 39.93 (NCH ₃), 180.43 (NCq)
11	124.62	123.65	129.95	128.81	148.73	128.81	129.95	123.65	124.62	117.62	153.26	117.62	-100.5	148.73	-	158.88	-238.2	-48.6	14.83 (SCH ₃), 19.23 (c CH ₃), 23.74 (tr CH ₃), 39.56 (NCH ₃), 173.76 (NCq)
12	125.32	119.55	128.23	114.15	138.26	113.75	128.48	119.60	126.46	120.18	79.26	120.84	-	138.56	-	165.81	-	-	20.08 and 24.67 (2 × CH ₃), 40.68 (NCH ₃), 49.68 (CH), 117.60 (CN), 168.73 (NCq)
13	127.07	118.63	127.90	113.15	138.81	113.85	125.48	119.33	127.72	122.94	80.23	119.77	-	137.86	-	166.43	-	-	19.90 (c CH ₃), 24.61 (tr CH ₃), 40.58 (NCH ₃), 51.48 (OCH ₃), 65.29 (CH) 168.75 (NCq), 169.34 (C=O)

N.b., Assignments within a row can be interchanged if italicized. ^a Values are given to only one decimal place for very broad signals or those measured indirectly; ¹³C referenced internally to TMS (0 ppm); ¹⁵N referenced externally to CH₃NO₂ (0 ppm). ^b Chemical shift questionable.

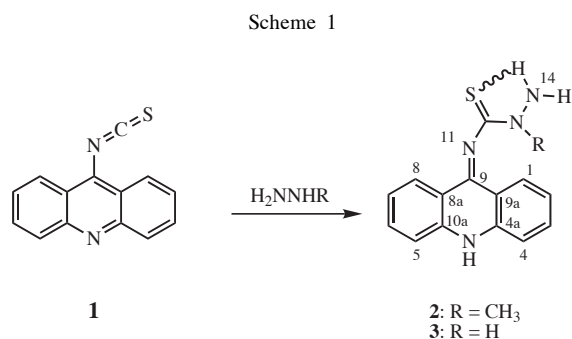
Table 2

¹H NMR chemical shifts^a (δ in ppm) and multiplicities^b for compounds **2–13** in DMSO-d₆ at 25 °C and calculated chemical shifts at the B3LYP/cc-pVTZ level of theory for all modeled structures.

	H-1	H-2	H-3	H-4	H-5	H-6	H-7	H-8	H-10	H-14	others
2 , exp	8.07, ddd	7.10, ddd	7.59, ddd	7.38, ddd	7.30, ddd	7.55, ddd	7.14, ddd	8.47, ddd	11.12, s	7.07, ^c s; 7.57, s	3.29 (3H, s, NCH ₃)
2	8.92 8.97	7.26 7.32	7.63 7.66	7.06 7.08	7.06 7.07	7.64 7.65	7.40 7.39	8.81 8.75	6.94 6.97	4.83, 3.83 4.07, 3.69	3.44 (NCH ₃) 3.77 (NCH ₃)
3 , exp	8.22, ddd	7.08, ddd	7.48, ddd	7.25, ddd	7.14, ddd	7.40, ddd	7.02, ddd	8.39, ddd	10.48, s	7.80, s; 8.15, s	10.01 (b s, H-13)
4 , exp	7.58, ddd	7.02, ddd	7.35, ddd	7.08, ddd	7.08, ddd	7.35, ddd	7.02, ddd	7.58, ddd	9.68, s	6.00, b s	3.15 (3H, s, NCH ₃), 9.49 (1H, s, H-11)
4	7.90 8.10	7.28 7.27	7.49 7.44	6.89 6.75	6.87 6.82	7.51 7.49	7.26 7.27	8.27 8.13	6.16 6.11	3.88 4.27	3.26 (NCH ₃), 5.33 (H-11) 3.25 (NCH ₃), 5.58 (H-11)
5 , exp	7.57, ddd	6.99, ddd	7.32, ddd	7.03, ddd	7.03, ddd	7.32, ddd	6.99, ddd	7.57, ddd	9.70, s	6.66, s	7.07 (m, H-4), 7.30 (m, H-3', H-5'), 7.77 (m, H-2', H-6), 10.06 (s, H-11) 6.97 (H-4), 7.15 (H-3', H-5'), 7.80 (H-2', H-6)
5 ₀	8.74	7.23	7.53	6.92	6.97	7.62	7.42	8.75	6.74	3.90, 6.88	7.31 (H-4), 7.49 (H-3', H-5'), 8.19 (H-2', H-6)
5	9.00	7.33	7.69	7.14	7.11	7.71	7.45	8.86	7.07	4.38, 4.15	7.18 (H-4), 7.36 (H-3', H-5'), 8.28 (H-2', H-6), 5.75 (H-11)
6 , exp	7.37, ddd	6.86, ddd	7.21, ddd	6.94, ddd	6.94, ddd	7.21, ddd	6.86, ddd	7.37, ddd	9.77, s	10.38, ^d s	6.64 (m, H-4), 6.90 (m, H-3', H-5'), 6.40 (m, H-2', H-6) 10.02 (1H, s, H-11)
6 ₀	9.03	7.39	7.71	7.15	7.01	7.56	7.05	7.90	7.10	5.64	7.08 (H-4), 7.46 (H-3', H-5'), 7.00 (H-2', H-6), 7.50 (H-13)
6	8.33	7.36	7.55	6.82	6.57	7.24	7.08	7.94	6.13	5.75 ^e	6.88 (H-4), 7.08 (H-3', H-5'), 6.87 (H-2', H-6), 5.91 (H-11) 2.86 (3H, s, NCH ₃)
7 , exp	8.45, ddd	7.06, ddd	7.50, ddd	7.31, ddd	7.25, ddd	7.49, ddd	7.09, ddd	8.43, dd	10.75, s	6.35, s	3.25 (NCH ₃)
7	9.53 9.56 7.80	7.25 7.29 7.26	7.62 7.63 7.48	7.04 7.05 6.89	7.05 7.06 6.85	7.64 7.64 7.49	7.37 7.37 7.26	8.89 8.88 8.38	6.90 6.91 6.15	4.91, 3.25 3.88, 3.15 3.81	3.38 (NCH ₃) 2.85 (NCH ₃), 4.06 (H-11)
7 _s	7.80	7.26	7.48	6.89	6.85	7.49	7.26	8.38	6.15	3.81	1.76, 1.89 (2 × 3H, b s, 2 × CH ₃), 3.54 (3H, b s, NCH ₃)
8	8.04, b s	7.16, b s	7.61, b s	7.42, b s	7.42, b s	7.61, b s	7.16, b s	8.04, b s	11.41, b s	-	1.32 (6H, s, 2 × CH ₃), 3.44 (3H, s, NCH ₃) 1.99 (b s, <i>trans</i> CH ₃), 2.05 (b s, <i>cis</i> CH ₃) 2.15 (b s, SCH ₃), 3.29 (s, NCH ₃) 1.69 (s, <i>trans</i> CH ₃), 1.75 (s, <i>cis</i> CH ₃) 2.17 (s, SCH ₃), 2.82 (s, NCH ₃) 2.04, 2.12 (2 × s, CH ₃), 3.40 (s, NCH ₃), 4.44 (s, CH)
9	8.18, ddd	7.65, ddd	7.88, ddd	8.23, ddd	8.23, ddd	7.88, ddd	7.65, ddd	8.18, ddd	-	6.86, s	2.04 (s, <i>trans</i> CH ₃), 2.10 (s, <i>cis</i> CH ₃), 3.04 (s, OCH ₃), 3.33 (s, NCH ₃), 4.02 (s, CH)
10	8.17, ddd	7.59, ddd	8.03, ddd	7.90, ddd	7.90, ddd	8.03, ddd	7.59, ddd	8.17, ddd	13.53, s	-	
11	7.96, ddd	7.46, ddd	7.74, ddd	8.00, ddd	8.00, ddd	7.74, ddd	7.46, ddd	7.96, ddd	-	-	
12	7.15, ddd	6.92, ddd	7.24, ddd	7.03, ddd	7.03, ddd	7.26, ddd	6.94, ddd	7.51, ddd	9.40, s	-	
13	7.28, ddd	6.80, ddd	7.16, ddd	6.91, ddd	6.97, ddd	7.19, ddd	6.88, ddd	7.17, ddd	9.25, s	-	

^a ¹H referenced internally to TMS (0 ppm). ^b Multiplicities are real observations in all cases; legend: m, multiplet; s, singlet; ^c Hydrogen bonded proton. ^d H-13.

product resulting from the reaction of **1** with hydrazine {4-(9,10-dihydroacridin-9-ylidene)thiosemicarbazide, **3**}. In the ^1H NMR spectrum of **3**, four distinct one-proton NH signals were observed, thus indicating that the N-14 pair of protons can indeed be non-equivalent. The likely explanation for this is that H-bonding to the sulfur atom by one of these Hs is occurring with resultant formation of a stable five-membered ring (see Scheme 1). Firm confirmation for the presence of an NH_2 group in the major species, and thus the formation of the N-13 methylated product **2**, was provided by an ^{15}N INEPT experiment whereby two hydrogen-bearing nitrogen atoms were readily observed, one of which was discerned to be NH and the other NH_2 . The distinctiveness of the two Hs bound to the same nitrogen was further emphasized by their disparate exchangeability evident from NOESY spectra, thus realizing their assignment.

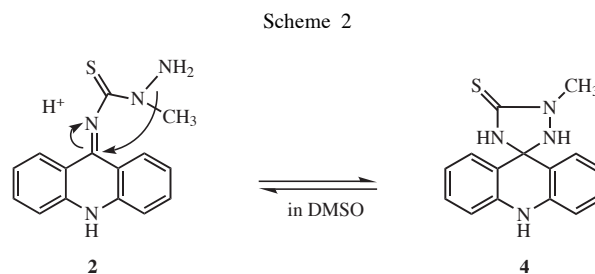


The reaction of acridin-9-yl isothiocyanate (**1**) with hydrazine or methylhydrazine results in products where the two hydrogens bound to N-14 are non-equivalent, presumably through hydrogen bonding to the thiocarbonyl. The reaction of methylhydrazine is highly regioselective yielding only the N-13 methylated product.

The minor species present in solution, rather than being the N-14 methylated product, was determined to be 2'-methyl spiro[9,10-dihydroacridinyl-9(10*H*)-5'-1',2',4'-triazolidine]-3'-thione (**4**, see Scheme 2). These two

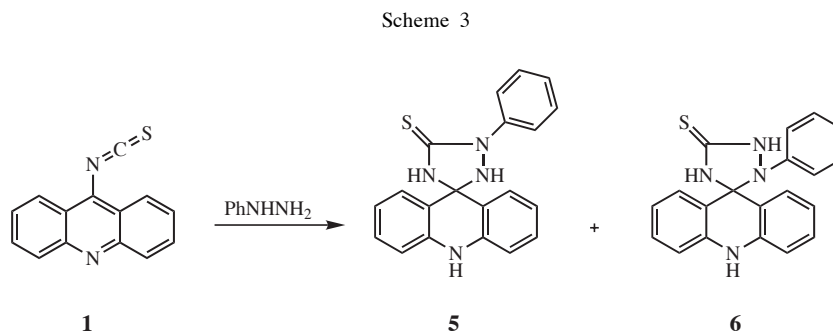
species, the open-chain form **2** and the spiro form **4**, were shown to be in dynamic exchange with each other by saturation transfer and variable temperature NMR. Curiously, there was no evidence for a spiro form present in the sample of **3**.

By contrast, the reaction of **1** with phenylhydrazine was generally not very regioselective (see Scheme 3), with the reaction in pyridine (45:55), ether (60:40), and methanol (70:30) media producing comparable mixtures of the two possible regioisomers (**5** and **6**, respectively); only in ethanol (90:10) did the reaction proceed with a strong bias towards the N-13 phenylated product **5**, *i.e.* the reaction proceeded *via* the α nitrogen similarly to methylhydrazine. Interestingly, for both regioisomers **5** and **6**, only the spiro form was evident in solution, and which serendipitously enabled their ready distinction based on appropriate NOEs between H-1/H-8, H-11, H-14, and the *ortho* protons of the phenyl ring.



The equilibrium between the open-chain (**2**, major species) and spiro (**4**, minor species) forms of the product from the reaction of methylhydrazine and **1**.

Treatment of **2** with mesitylnitrile oxide quantitatively provided semicarbazide **7** (see Scheme 4) with an intensive IR band at 1680 cm^{-1} , thereby confirming the presence of a C=O group. The oxo analogue not only broadens the range of compounds examined, but was itself of interest for fluorescence and bioactivity

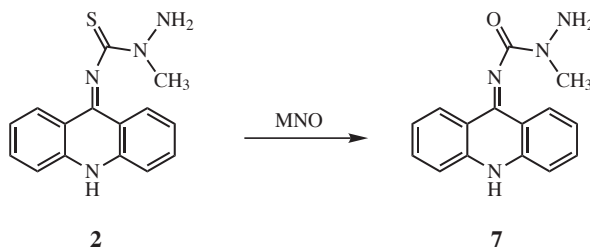


The reaction of **1** with phenylhydrazine was not regioselective with both regioisomers being produced in a variety of solvents. Only in ethanol did the N-13 phenylated product predominate in the reaction mixture. Interestingly, for both compounds, the spiro forms of each were predominant in solution with no evidence of the open-chain forms.

evaluations and which is more conveniently prepared in any case from its thio analogue. Although inversion at N-11 is slow enough to render the outer acridinyl rings distinct—the same as for the thio analogue—the process, however, is sufficiently fast at ambient temperature such that under normal NOE equilibrium conditions, equivalent enhancements are observed for H-1 and H-8 upon irradiation of the N-methyl protons, thus in contrast to the

81.78 ppm and the correlations of the equivalent *geminal* methyl protons to the two sp^3 -hybridized nitrogen atoms, N-11 and N-14. With both the labile H-14 proton (located based on its correlations to Cq and the *geminal* methyl carbons) and the NCH_3 protons showing correlations (HMBC, HSQC as appropriate) to both N-13 and N-14, the structure, though surprising, was without doubt as depicted in Scheme 5. This same pair of products could

Scheme 4



Reaction of **2** with mesitylnitrile oxide (MNO) provided semicarbazide **7** in quantitative yield.

thio analogue. Furthermore, the NH_2 protons resonated as one signal, presumably due to a lack of hydrogen bonding to the oxygen. Also distinctive for this compound was the absence of a spiro form in solution. But in addition to providing a useful substrate for further bioactivity and fluorescence studies, the compound also enabled the observation of all nitrogen atoms present, which was highly useful for comparative purposes with other structures.

The manufacture of **7** also provided the opportunity to compare the anisotropic effects on H-1 and H-8 of thiocarbonyl vs carbonyl. The calculated differences (*vide infra*) between H-1 and H-8 (+0.17 and +0.66 ppm for **2** and **7**, respectively) did not compare that well with the experimental values (−0.40 and +0.02 ppm, respectively), but comparison of the two H-8s revealed that H-8 in **2** should be shielded by 0.12 ppm relative to H-8 in **7**, which compares well with the experimental difference of −0.04 ppm. Comparison of the two H-1s revealed that H-1 in **2** should be shielded by 0.60 ppm relative to its counterpart in **7**, but in fact is shielded by 0.38 ppm, this infers that there are additional factors, *e.g.* steric, in play and which differ fundamentally between the two structures.

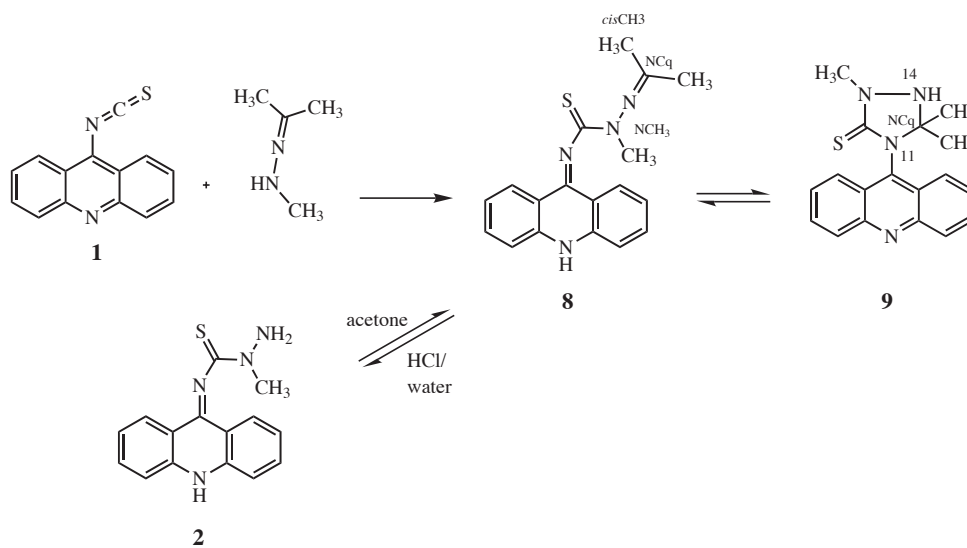
Acridin-9-yl isothiocyanate (**1**) was reacted with acetone 2-methyl hydrazone to provide the expected 1-isopropylidene-2-methylthiosemicarbazide **8**, but only as a minor species (25%), with the majority of the product preferentially adopting the cyclic **9**. The evidence for the new ring in **9** stemmed from the chemical shift of NCq at

also be produced by the reaction of **2** with acetone, and indeed the structure of the **8/9** products from **1**/acetone 2-methyl hydrazone was also proven by hydrolysis back to the thiosemicarbazide **2**.

For **8**, although a dihydroacridine moiety was apparent, the outer rings of this moiety only provided an averaged set of signals in $DMSO-d_6$ wherein all signals were exchange broadened, some considerably, due to the relatively fast inversion at N-11 despite the placement of the labile proton on N-10. The symmetric-site interconversion rate, however, was slow enough in $CDCl_3$ solution (where the disparity of the two species became even more pronounced at 9:1) to observe the non-equivalence of the off-central axis nuclei of the acridine moiety. Interestingly, a feature of the system in DMSO was the slow interconversion between **8** and **9**. Of note also, the determination of the structure of **9** is consistent with, or even substantiates, the structure of **2** whereby the alternative regioisomer possessing methyl on the N14 nitrogen is discounted by the correlation from H-14 to the *geminal* methyl carbon atoms.

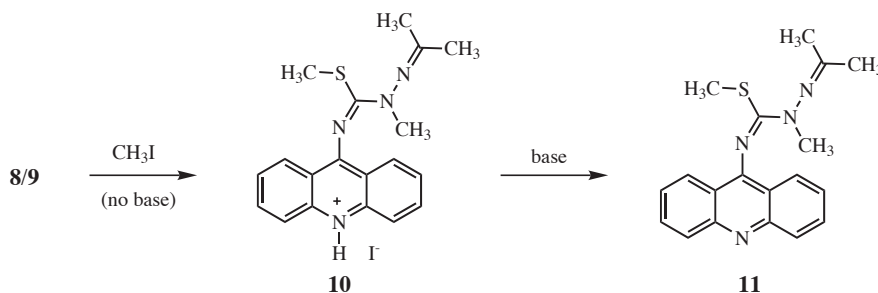
To examine if spiro cyclization could be effected with the reaction pathway of N-14 blocked, **8/9** was treated with methyl iodide without base (noting other observations [9]), though this opened up the possibility of reaction at NCq given the behavior of **8/9**. Nonetheless, the reaction afforded *S*-methyl isothiosemicarbazide hydroiodide **10**, the expected *S*-methylated structure with the labile H maintained on N-10 (see Scheme 6). Of note is that the cyclic **9** need not necessarily revert to the open-

Scheme 5



Reaction of **1** with acetone 2-methyl hydrazone yielded the expected structure, **8**, but only as a minor component of the solution mixture, the majority adopting the cyclic structure **9**. The reversible reaction of **2** with acetone provided the same pair of products.

Scheme 6



Reaction of **8/9** with CH_3I yielded the expected S-methylated structure. Although unquestionably it is the conventional structure with a single positive charge on N-10 that is the major contributing canonical form to **10**, other canonical forms could also contribute to account for various observed traits of the compound (see text). Treatment with base provided the expected structure **11** without the need to invoke mesomeric forms to rationalise observed features.

chain form (**8**) to undergo nucleophilic reaction with CH_3I (or other electrophilic reagents) *via* the sulfur atom as this could conceivably be part of a concerted process yielding **10** directly (see Figure 1). Thus, it is probably inconsequential that the exchange rate between **8** and **9** was relatively slow and could not be observed by NMR experiments sensitive to exchange processes in contrast to the case of **2** and **4** which could be observed to undergo exchange.

Although unquestionably it is the conventional structure with a single positive charge on N-10 that is the major contributing canonical form to **10**, other canonical forms may be contributing as implied by some of the observed traits of the compound: the deshielding of NCq (180.43 ppm) and C-12 (165.87 ppm), the

shielding of N-11 (-147.0 ppm), and the evident dynamics. For example, double bond character for the $\text{C}_{12}\text{-S}$ bond would account for restricted movement for the S-methyl and consequently the breadth of the C-12

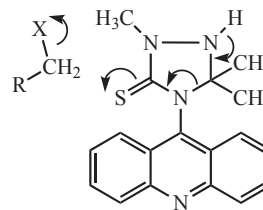
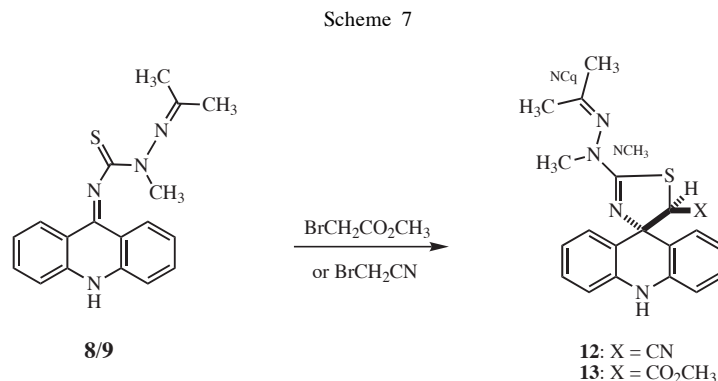


Figure 1. The reaction of **9** with an electrophile can conceivably undergo reaction *via* attack by the sulfur atom concomitant with ring opening.

and SCH_3 signals. Similarly, the breadth of the *geminal* methyls in the 1H NMR suggests limited rotation about the C_q-N_{14} bond, though the inequality of the line broadening implies an additional exchange process is also affecting these methyls. This is also further emphasized by the breadth of the C-9 and NC_q signals. Treatment with base readily provided the expected structure **11** without the need to consider the contribution of mesomeric forms to rationalise the observed features.

Alkylation–cyclization reactions of **8/9** with bifunctional electrophiles bromoacetonitrile and methyl bromoacetate afforded the readily recognisable spirocycles **12** and **13**, respectively, without any sign of an NC_q -attacked product in either case (Scheme 7). In the 1H NMR spectra of both **12** and **13**, the CH_2 signal was lacking and instead, a CH signal was present at 4.2–4.4 ppm together with a quaternary carbon (C-9) at 78–79 ppm.



The reaction of **8/9** with bifunctional electrophiles, induced, not unexpectedly given the blocking of N-13, spiro products. These two reactions demonstrate the susceptibility of C-9 to nucleophilic attack and the formation of spiro products, though even given such propensity, it is still surprising that the NC_q carbon is not attacked by the methylene carbon of the reagent to form a non-spiro six-membered ring.

To provide additional support for the elucidated structures, density functional theory (DFT) calculations were performed for structures **2**, **4**, **5** and **6** (together with their open-chain forms), and **7** (together with its spiro form) whereby the geometries were optimized at the B3LYP/6-31G(d,p) level of theory and the fractional charges and chemical shifts calculated at the B3LYP/cc-pVTZ level of theory. Unfortunately, the gas-phase energies (Table 3) did not correlate well with the solution-state energies, and this is not unexpected for polar molecules if solvation is not taken into account. However, accurate solution-state determination of the energies was not the principal target of the calculations which would have proven exorbitantly expensive if full inclusion of the solvent had been undertaken and it was thus considered to be outside the scope of this current work and capacity of the available computer facility. Of

Table 3
Energies of all modeled structures at the B3LYP/6-31G(d,p) level of theory

structure ^a	conformer	energy (kcal/mol)	comments
2	1	1.72	open-chain form
	2	3.51	
4	1	0.00	spiro form
	2	1.65	
5o	1	2.26	open-chain form
	2	5.93	
5	1	0.00	spiro form
6o	1	0.00	open-chain form
6	1	4.44	spiro form
7	1	1.06	open-chain form
	2	1.63	
7s	1	0.00	spiro form

^a An appended “o” or “s” indicates an open-chain or spiro form, respectively, of the associated structure.

the comparison of three sets of open-chain forms vs their spiro counterparts, only once was the correct form predicted. This insinuates the large role that solvation plays in determining the most stable structure and this has been an aspect that has been underappreciated in assessing the stability of the spiro forms in reversible cases.

However, the primary aim of the calculations was to verify the structures by calculation of the chemical shifts (see Tables 1 and 2). Other than the chemical shifts of the labile protons, adequate agreement was found for support for the gross chemical structures. The results, however, lacked the high fidelity anticipated and the presence of various contributing tautomers, conformational equilibria, and extensive hydrogen bonding as a result of the high polarity of the compounds all conspired to degrade the quality of the final results.

EXPERIMENTAL

See succeeding article [6] for general details.

Computational Methods.

Geometry optimizations were performed using a DFT [24] method contained within the Gaussian 98 program [25]; a B3LYP functional [26] and a 6-31G(d,p) basis set [27] with a tight SCF convergence criterion were used in order to define the structures at a sufficiently high level of theory appropriate [28] for the calculation of reliable NMR shielding constants. The energies of the structures were calculated at the same level of theory. For calculation of the chemical shifts, nuclear shieldings were calculated using the Gaussian 98 program [25] implementation of the GIAO method [29] at the B3LYP/cc-pVTZ level [30] of theory. (Atomic charges were also calculated at this same level of theory.) The isotropic magnetic shieldings (σ) so obtained were evaluated as chemical shifts (δ) based on their difference from the shieldings of TMS (for both ^{13}C and ^1H) or nitromethane (for ^{15}N), accordingly. For calibration of the chemical shifts (^{15}N , ^{13}C , and ^1H), a range of different compounds (*ca.* 25 in total) were selected encompassing various structural entities and included several substituted pyridines and pyrimidines, several substituted benzenes, naphthalene and other condensed systems, furan, *N,N*-dimethyl formamide, nitromethane, TMS, and the aliphatic heterobicyclic systems of refs [31,32] amongst others. The geometries of the selected calibration compounds were optimized and their chemical shifts calculated using the same methodology as for the compounds under study; their experimental chemical shifts were taken from refs [28,31–33] and plotted *vs* the calculated shifts, hence the relationships [33]:

$$\begin{aligned}\delta_{\text{C}} &= 0.9980 \text{ } _{-} (\sigma_{\text{TMS}} - \sigma_{\text{C}}) - 3.780 \text{ } (R^2 = 0.9987) \\ \delta_{\text{H}} &= 0.9736 \text{ } _{-} (\sigma_{\text{TMS}} - \sigma_{\text{H}}) + 0.058 \text{ } (R^2 = 0.9970) \\ \delta_{\text{N}} &= 0.9099 \text{ } _{-} (\sigma_{\text{nitro}} - \sigma_{\text{N}}) - 10.743 \text{ } (R^2 = 0.9922)\end{aligned}$$

This methodology has been found [32,33,34] to be a reliable and useful method for the calculation of chemical shifts for ^{15}N , ^{13}C , and ^1H nuclei in terms of structural determination.

4-(9,10-Dihydroacridin-9-ylidene)-2-methylthiosemicarbazide (**2**) [35] and 2'-Methyl spiro[dihydroacridinyl-9(10*H*)-5'-1',2',4'-triazolidine]-3'-thione (**4**).

To a solution of acridin-9-yl isothiocyanate [36] (**1**, 1 g, 4.2 mmol) in methanol (15 mL), methylhydrazine (0.194 g, 0.223 mL, 4.2 mmol) was added dropwise at 0 °C. The mixture was allowed to stir at room temperature in an atmosphere of nitrogen for 2 h until completion of the reaction as indicated by TLC (benzene/acetone 5:2). The solvent was then removed under vacuum, ether added, and the precipitate collected by filtration and dried to provide a mixture of products **2** and **4** as a yellow powder (solution equilibrium by ^1H NMR 96:4). Yield 85%; mp 215–217 °C (presumably for **2** and **4**, methanol); Found: C, 64.02; H, 4.79; N, 20.02. $\text{C}_{15}\text{H}_{14}\text{N}_4\text{S}$ requires C, 63.80; H, 5.00; N, 19.84%; ν_{max} (KBr)/ cm^{-1} 1621, 1573, 1471, 1359, 1252, 1154, 1021, 959, 758, 622.

4-(9,10-Dihydroacridin-9-ylidene)thiosemicarbazide (**3**) [35].

To a solution of acridin-9-yl isothiocyanate [36] (**1**, 1 g, 4.2 mmol) in methanol (15 mL) hydrazine hydrate (0.210 g, 0.204 mL, 4.2 mmol) was added dropwise at 0 °C. The mixture was

allowed to stir at room temperature under nitrogen for 2 h until completion of the reaction (monitored by TLC, benzene/acetone 5:2). The solvent was then removed under vacuum, ether added, and the precipitate collected by filtration and dried to provide **3**. Yield 70%; mp 210 °C (methanol); Found: C, 62.53; H, 4.29; N, 20.47. $\text{C}_{14}\text{H}_{12}\text{N}_4\text{S}$ requires C, 62.66; H, 4.51; N, 20.88%.

2'-Phenyl spiro[dihydroacridinyl-9(10*H*)-5'-1',2',4'-triazolidine]-3'-thione (**5**).

Acridin-9-yl isothiocyanate [36] (**1**, 1 g, 4.2 mmol) was dissolved in ethanol (15 mL) and to this solution was added phenylhydrazine (0.453 g, 0.413 mL, 4.2 mmol). The reaction was then left to stir for 2 h at room temperature under nitrogen. The solvent was evaporated and the resulting precipitate washed with ether and then dried to provide a mixture of products **5** and **6** in a ratio of 90:10 (by ^1H NMR). Product **5** was separated by column chromatography (cyclohexane/acetone 2:1) and crystallized from ethanol. Yield 68%; mp 273 °C (ethanol); Found: C, 69.58; H, 4.64; N, 16.01. $\text{C}_{20}\text{H}_{16}\text{N}_4\text{S}$ requires C, 69.74; H, 4.68; N, 16.27%.

1'-Phenyl spiro[dihydroacridinyl-9(10*H*)-5'-1',2',4'-triazolidine]-3'-thione (**6**).

Acridin-9-yl isothiocyanate [36] (**1**, 1 g, 4.2 mmol) was dissolved in pyridine (15 mL) at room temperature and to this solution was added phenylhydrazine (0.453 g, 0.413 mL, 4.2 mmol). The reaction was then left to stir for 2 h at room temperature under nitrogen. The precipitate was collected by filtration, washed with ether, and then dried to provide a mixture of products **6** and **5** in a ratio of 55:45 (by ^1H NMR). Product **6** was separated by column chromatography (cyclohexane/acetone 2:1). Yield 58%; mp 278 °C; Found: C, 69.52; H, 4.58; N, 15.88. $\text{C}_{20}\text{H}_{16}\text{N}_4\text{S}$ requires C, 69.74; H, 4.68 N, 16.27%.

4-(9,10-Dihydroacridin-9-ylidene)-2-methylsemicarbazide (**7**).

Thiosemicarbazide **2** (0.423 g, 1.5 mmol) was dissolved in dry acetonitrile (10 mL) and to this solution was added mesitylnitrile oxide (0.240 g, 1.5 mmol). The mixture was allowed to stir for 3 h at room temperature following which the solvent was concentrated under reduced pressure and the resulting solid collected by filtration, dried, and crystallized from methanol. Yield 80%; mp 274–276 °C (methanol); Found: C, 68.03; H, 4.94; N, 20.87. $\text{C}_{15}\text{H}_{14}\text{N}_4\text{O}$ requires C, 67.65; H, 5.30; N, 21.04%; ν_{max} (KBr)/ cm^{-1} 1680; *m/z*: 821 ($3\text{M}^+ + \text{Na}$, 1%), 799 ($3\text{M}^+ + \text{H}$, 2), 555 ($2\text{M}^+ + \text{Na}$, 9), 533 ($2\text{M}^+ + \text{H}$, 23), 289 ($\text{M}^+ + \text{Na}$, 21), 267 ($\text{M}^+ + \text{H}$, 100), 250 ($\text{M}^+ + \text{H} - \text{NH}_3$, 48), 235 ($250 - \text{CH}_3$, 4), 222 ($250 - \text{CO}$, 20), 195 (30), 194 (acridine + NH, 44), 193 (20), 179 (5); $J_{\text{H,H}}$ couplings (Hz) extracted by spin simulation [23]: $J_{\text{H}_1,\text{H}_2} = 8.4$, $J_{\text{H}_1,\text{H}_3} = 1.4$, $J_{\text{H}_1,\text{H}_4} = 0.6$, $J_{\text{H}_2,\text{H}_3} = 7.0$, $J_{\text{H}_2,\text{H}_4} = 1.2$, $J_{\text{H}_3,\text{H}_4} = 8.3$, $J_{\text{H}_5,\text{H}_6} = 8.2$, $J_{\text{H}_5,\text{H}_7} = 1.1$, $J_{\text{H}_5,\text{H}_8} = 0.6$, $J_{\text{H}_6,\text{H}_7} = 7.0$, $J_{\text{H}_6,\text{H}_8} = 1.5$, $J_{\text{H}_7,\text{H}_8} = 8.2$.

4-(9,10-Dihydroacridin-9-ylidene)-1-isopropylidene-2-methylthiosemicarbazide (**8** and **9**) [35].

Acridin-9-yl isothiocyanate [36] (**1**, 0.5 g, 2.1 mmol) was dissolved in acetone (15 mL) and to this solution was added *N*-isopropylidene-*N'*-methylhydrazine (0.189 g, 2.2 mmol) and the mixture left to stir 1 h at room temperature. The solvent was then evaporated under reduced pressure, ether added and the precipitant collected by filtration, dried, and crystallized from methanol to give a mixture of **8** and **9** (solution equilibrium by

¹H NMR: 3:1 in DMSO-d₆ and 9:1 in CDCl₃, respectively). Yield 65%; mp 184–187 °C (presumably for **8** and **9**, methanol); Found: C, 67.09; H, 5.44; N, 17.32. C₁₈H₁₈N₄S requires C, 67.05; H, 5.63; N, 17.38%.

Hydrolysis of **8/9**.

A mixture of **8/9** (0.2 g, 0.6 mmol) was dissolved in methanol (10 mL) and treated with 6 N HCl. The mixture was refluxed for 2 h and then poured into ice/water. The precipitate was collected by filtration, dried and again dissolved in methanol and the pH of the solution adjusted to 7 using sodium methoxide. The solvent was then partially evaporated off, ether added and the resulting precipitate collected by filtration, dried, and crystallized from methanol. Yield 65%; mp 143 °C (methanol); Spectral data were identical to the spectral data of **2**.

S-Methyl 4-(acridin-9-yl)-1-isopropylidene-2-methyl isothiosemicarbazide hydroiodide (**10**).

A solution of **8/9** (0.4 g, 1.2 mmol) in dry acetonitrile (15 mL) was treated with CH₃I (0.187 g, 0.082 mL, 1.32 mmol). The mixture was allowed to stir for 5 h at room temperature during which a yellow precipitate had formed. The product was isolated by filtration, washed with ether, and then dried to give a yellow solid **10**. Yield 70%; mp 166–168 °C; Found: C, 48.75; H, 4.68; N, 12.37. C₁₉H₂₁N₄S requires C, 49.14; H, 4.56; N, 12.07%.

S-Methyl 4-(acridin-9-yl)-1-isopropylidene-2-methyl isothiosemicarbazide (**11**).

To a suspension of **8/9** (0.5 g, 1.55 mmol) and anhydrous potassium carbonate (0.321 g, 2.35 mmol) in dry acetonitrile (15 mL), iodomethane (0.22 g, 0.096 mL, 1.55 mmol) was added dropwise and the mixture left to stir for 3 h at room temperature. The solvent was evaporated and the resulting precipitate washed with ether, dried, and crystallized from methanol. Yield 60%; mp 154 °C (methanol); Found: C, 67.63; H, 5.75; N, 16.74. C₁₉H₂₀N₄S requires C, 67.83; H, 5.99; N, 16.65%.

5'-Cyano-2'-(2-isopropylidene-1-methylhydrazinyl)-spiro[dihydroacridinyl-9(10H)-4'(5'H)-1',3'-thiazoline] (**12**).

Thiosemicarbazide **8/9** (0.322 g, 1 mmol) was dissolved in methanol (10 mL) and to this solution bromoacetonitrile (0.132 g, 0.074 mL, 1.1 mmol) was added dropwise followed by sodium methoxide (0.054 g, 1 mmol). The reaction was then left to stir for 4 h at room temperature under nitrogen. The obtained solid **12** was filtered off, washed with ether, dried, and then crystallized from a mixture of methanol/diethyl ether. Yield 72%; mp 106 °C (methanol/diethyl ether); Found: C, 66.20; H, 5.28; N, 19.14. C₂₀H₁₉N₅S requires C, 66.46; H, 5.30; N, 19.37%.

5'-Methoxycarbonyl-2'-(2-isopropylidene-1-methylhydrazinyl)-spiro[dihydroacridinyl-9(10H)-4'(5'H)-1',3'-thiazoline] (**13**).

To a solution of **8/9** (0.322 g, 1 mmol) in methanol (10 mL) was added methyl bromoacetate (0.168 g, 0.101 mL, 1.1 mmol) followed by sodium methoxide (0.06 g, 1.1 mmol) after which the solution left to stir for 5 h at room temperature under nitrogen. The solid was collected by filtration, washed with ether, and then dried to provide **13**. Yield 70%; mp 126 °C (methanol); Found: C, 63.72; H, 5.57; N, 13.84. C₂₁H₂₂N₄O₂S requires C, 63.94; H, 5.62; N, 14.20%.

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REFERENCES AND NOTES

- *) Part 1. For Part 2 see the succeeding article (ref [6]). **) Corresponding author.
- [1] W. A. Denny, *Anticancer Drug. Des.*, **4**, 241–263 (1989).
 - [2] M. Demeunynck, F. Charmantray and A. Martelli, *Curr. Pharm. Design*, **7**, 1703–1724 (2001).
 - [3] M. F. Braña, M. Cacho, A. Gradillas, B. de Pascual-Teresa and A. Ramos, *Curr. Pharm. Design*, **7**, 1745–1780 (2001).
 - [4] M. J. Robinson and N. Osheroff, *Biochemistry*, **29**, 2511–2515 (1990).
 - [5] T. A. Gourdie, A. S. Prakash and W. A. Denny, *J. Med. Chem.*, **34**, 240–248 (1991).
 - [6] E. Balentová, J. Imrich, J. Bernát, L. Suchá, M. Vilková, N. Prónayová, P. Kristian, K. Pihlaja and K. D. Klika, *J. Heterocyclic Chem.*, **43**, 645 (2006).
 - [7] K. D. Klika, J. Imrich, M. Vilková, J. Bernát and K. Pihlaja, *J. Heterocyclic Chem.*, **43**, 739 (2006).
 - [8] K. D. Klika, J. Bernát, J. Imrich, I. Chomča, R. Sillanpää and K. Pihlaja, *J. Org. Chem.*, **66**, 4416–4418 (2001).
 - [9] K. D. Klika, E. Balentová, J. Bernát, J. Imrich, E. Kleinpeter, A. Koch and K. Pihlaja, submitted to *Chem. Eur. J.*, (2005).
 - [10] P. Kristian, J. Bernát, J. Imrich, I. Danihel, G. Suchár, S. Hočová, T. Bušová, J. Guspanová and A. Linden, *Molecules*, **1**, 181–189 (1996).
 - [11] P. Kristian, I. Chomča, J. Bernát and J. Imrich, *Chem. Papers*, **53**, 49–52 (1999).
 - [12] P. Kristian, J. Bernát, J. Imrich, E. Sedlák, J. Alföldi and M. Čornanič, *Heterocycles*, **55**, 279–290 (2001).
 - [13] J. Bernát, I. Chomča, P. Kristian, K. Pihlaja, K. D. Klika and J. Imrich, *Heterocycles*, **51**, 137–140 (1999).
 - [14] P. Kristian, E. Balentová, J. Bernát, J. Imrich, E. Sedlák, I. Danihel, S. Böhm, N. Prónayová, K. D. Klika, K. Pihlaja and J. Baranová, *Chem. Pap.*, **58**, 268–275 (2004).
 - [15] J. Bernát, E. Balentová, P. Kristian, J. Imrich, E. Sedlák, I. Danihel, S. Böhm, N. Prónayová, K. Pihlaja and K. D. Klika, *Coll. Czech. Chem. Commun.*, **69**, 833–849 (2004).
 - [16] K. D. Klika, L. Janovec, J. Imrich, G. Suchár, P. Kristian, R. Sillanpää and K. Pihlaja, *Eur. J. Org. Chem.*, 1248–1255 (2002).
 - [17] K. D. Klika, P. Valtamo, L. Janovec, G. Suchár, P. Kristian, J. Imrich, H. Kivelä, J. Alföldi and K. Pihlaja, *Rapid Commun. Mass Spectrom.*, **18**, 87–95 (2004).
 - [18] B. Adcock, in *Aminoacridines in Acridines*, ed. R. M. Acheson (from *The Chemistry of Heterocyclic Compounds*, ed. A. Weissberger and E. C. Taylor), J. Wiley, New York, 1973, pp 109–140.
 - [19] F. Duus, in *Thioureas and Thiosemicarbazides in Comprehensive Organic Chemistry* (ed. D. Barton and W. D. Ollis), Pergamon Press, Oxford, 1979, pp 452–461.
 - [20] U. Kraatz, in *Thiosemicarbazide in Methoden der Organischen Chemie (Houben-Weyl)*, G. Thieme Verlag, Stuttgart, 1983, vol. 4, pp 506–515.
 - [21] K. A. Jensen, U. Anthoni, B. Kägi, Ch. Larsen and C. Th. Pedersen, *Acta Chem. Scand.*, **22**, 1–50 (1968).

- [22] M. U. Roslund, P. Virta and K. D. Klika, *Org. Lett.*, **6**, 2673–2676 (2004).
- [23] See for example: R. Laatikainen, M. Niemitz, U. Weber, J. Sundelin, T. Hassinen and J. Vepsäläinen, *J. Magn. Reson. Ser. A*, **120**, 1–10 (1996). See also: *Peak Research NMR Software*, Perch Solutions Ltd., Kuopio, Finland, 2003 (<http://www.perchsolutions.com>).
- [24a] P. Hohenberg and W. Kohn, *Phys. Rev. B*, **136**, 864–871 (1964); [b] W. Kohn and L. J. Sham, *Phys. Rev. A*, **140**, 1133–1138 (1965); [c] R. G. Parr and W. Yang, in *Density Functional Theory Of Atoms And Molecules*, Oxford Science Publishers, New York, 1989.
- [25] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, N. Rega, P. Salvador, J. J. Dannenberg, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, A. G. Baboul, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S. Replogle and J. A. Pople, *Gaussian 98*, Revision A.11.3, Gaussian, Inc., Pittsburgh, PA, 2002.
- [26a] A. D. Becke, *Phys. Rev. A*, **38**, 3098–3100 (1988); [b] A. D. Becke, *J. Chem. Phys.*, **98**, 5648–5652 (1993); [c] C. Lee, W. Yang and R. G. Parr, *Phys. Rev. B*, **37**, 785–789 (1988); [d] B. Miehlich, A. Savin, H. Stoll and H. Preuss, *Chem. Phys. Lett.*, **157**, 200–206 (1989).
- [27] W. J. Hehre, L. Radom, P. v. R. Schleyer and J. A. Pople, in *Ab Initio Molecular Orbital Theory*, Wiley, New York, 1986.
- [28] A. Bagno, *Chem. Eur. J.*, **7**, 1652–1661 (2001).
- [29] K. Wolinski, J. F. Hinton and P. Pulay, *J. Am. Chem. Soc.*, **112**, 8251–8260 (1990).
- [30] R. A. Kendall, T. H. Dunning Jr. and R. J. Harrison, *J. Chem. Phys.*, **96**, 6796–6806 (1992).
- [31] P. Tähtinen, J. Sinkkonen, K. D. Klika, V. Nieminen, G. Stájer, Zs. Szakonyi, F. Fülöp and K. Pihlaja, *Chirality*, **14**, 187–198 (2002).
- [32] P. Tähtinen, A. Bagno, K. D. Klika and K. Pihlaja, *J. Am. Chem. Soc.*, **125**, 4609–4618 (2003).
- [33] J. Mäki, P. Tähtinen, L. Kronberg and K. D. Klika, *J. Phys. Org. Chem.*, **18**, 240–249 (2005).
- [34] K. D. Klika, J. Imrich, I. Danihel, S. Böhm, P. Kristian, S. Hamul'aková, K. Pihlaja, A. Koch and E. Kleinpeter, *Magn. Reson. Chem.*, **43**, 380–388 (2005).
- [35] Compounds **2**, **3**, and **8** (and hence also **4** and **9**) appear to have been reported previously, D. Mayer, G. Hermann and K. Sasse, German Patent DE 1811409 19700618 (1970), though without full structural characterization.
- [36] D. Mazagová, D. Sabolová, P. Kristian, J. Imrich, M. Antalík and D. Podhradský, *Coll. Czech. Chem. Commun.*, **59**, 203–212 (1994).