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Synthesis and RNase A inhibition study of C_2-symmetric bis-isochromenyl sulfones

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ABSTRACT

A new class of C_2-symmetric bis-isochromene derivatives with 3,3′-linkage has been synthesized from bis-propargyl sulfones. The method involves treatment of the sulfones with triethylamine to form the isochromene derivatives presumably via the intramolecular Michael addition to the intermediate bis-allenic sulfones. Interestingly, the product expected from the Garratt–Braverman pathway was not obtained. The bis-isochromene 7d displayed RNase A inhibition activity, much stronger than the isochromene **8** and bis-isocoumarin **9**.

(1*H*)-Isochromen-2-ones, better known as isocoumarins, have drawn considerable interest as a wide variety of natural and unnatural products containing such moieties are endowed with a range of biological activities.[1] Moreover, isocoumarins serve as useful and versatile synthetic intermediates.[2] In recent past, the activity of isocoumarins as potent anticancer agents has generated keen interest in these molecules and their analogues.[3] Because of the similarity in chemical and biological activities between the oxygen species and their nitrogen counterparts, (1*H*)-isochromen-1-imine analogues have also drawn interest especially their synthesis[4] in recent years. It occurred to us that bis-isochromen-1-imine derivatives with a C_2-symmetry might be even more attractive as many of the biological targets like homodimeric proteases[5] have similar symmetry elements. ds-DNA also has a pseudo C_2-symmetry and thus interactions with other C_2-symmetric molecules can be expected to be stronger. Literature survey revealed that till now, there is no report of synthesis of bis-(1*H*)-isochromen-1-imines. Herein, we report a near quantitative synthesis of such imines with C_2-symmetry starting from bis-propargyl sulfones. It may be noted that all the current methods available for the synthesis of isochromenones involve a metal ion-catalyzed intramolecular addition of amide oxygen to an acetylenic functionality. For example, Lieu et al.[4a] have recently reported a $AgSbF_6$-mediated intramolecular enyne–amide cyclization for the synthesis of isochromenoneimines. The method requires 25 mol % catalyst and refluxing in THF at 80 °C for 8 h. In this year, Ma et al.[4b] reported that the same reaction can be carried out using 5 mol % of AgOTf and refluxing in dichloroethane at 60 °C for 2.5 h. On the contrary, the present method is mild, quantitative, rapid (over within minutes) and easy to execute (no requirement of metal ion, requires only 1.0 equiv of Et_3N).

Before embarking upon any synthetic endeavour, we considered the possible reaction pathways of *o*-amido bis-allenic sulfones, derivable from the corresponding bis-propargyl sulfones[6] of the type **A** (Scheme 1). The allenic sulfone can undergo Garratt–Braverman cyclization[7] via conformation **B** to form the sulfolene **D** while intramolecular nucleophilic addition of amide oxygen or nitrogen involving conformation **C** will lead to bis-isochromene imines **E** or bis-isoquinolines **F**.

With this background, we proceeded to synthesize the starting materials **1a–d**. Thus EDC-mediated coupling of 2-iodo benzoic acid with amino acid benzyl esters produced the aminoacyl derivatives. Sonogashira coupling[8] with propargyl alcohol followed by mesylation (mesyl chloride, Et_3N) and bromination (LiBr, THF)[9] gave the bromide. The latter upon treatment with Na_2S/TBAB in THF–water afforded the sulfide **2a–d** which on oxidation with *m*CPBA produced the sulfone (Scheme 2). The simple aryl sulfone **3** was similarly prepared from the intermediate bromide **6d**.

With the starting bis-propargyl sulfones in hand, the stage was set to check their reactivity under basic conditions (Scheme 3). As an initial experiment, the sulfone **1d** was dissolved in $CDCl_3$ in an NMR tube and 1.0 equiv amount of triethylamine was added and the ^1H NMR was recorded again at different time points (Fig. 1). We are amazed to see that within 2 min (the time taken to record the spectrum after the addition of Et_3N), the AB quartet for the methylenes adjacent to the sulfonyl in the substrate at $\sim\delta$ 4.04 was completely replaced by a similar AB quartet at $\sim\delta$ 3.6 pair of doublets (J = 14.2 Hz). At the same time, a new singlet appeared at $\sim\delta$ 6.1 which was assigned to the 4 and 4′ hydrogens of isochromen-1-imine.[10] After that there was no change in the spectrum pointing out that the reaction was complete within the first few minutes. It was repeated on a larger scale in $CHCl_3$ and Et_3N and the product was isolated pure in >95% yield by column chromatography over silica gel using hexane–EtOAc (1:1) as an eluent. That the product was not a result of Garratt–Braverman cyclization

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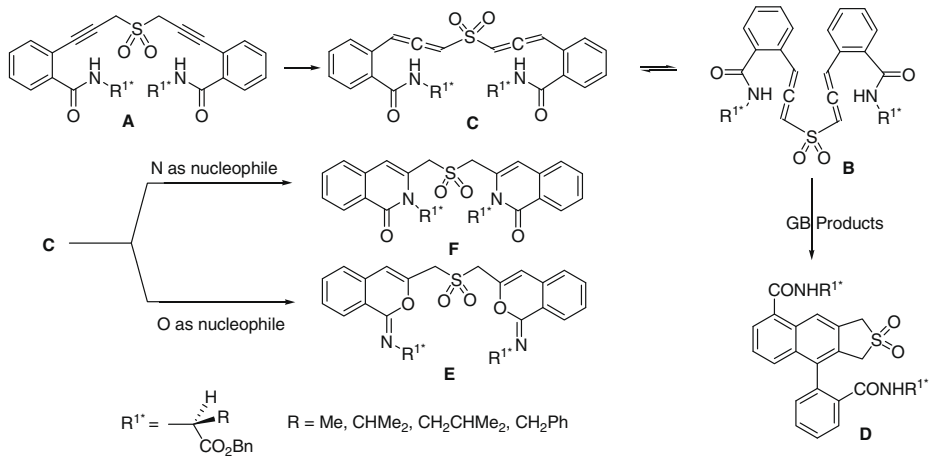
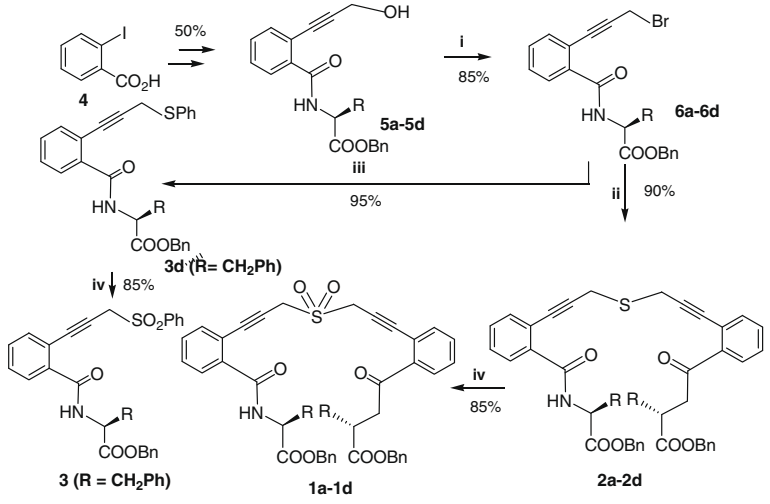


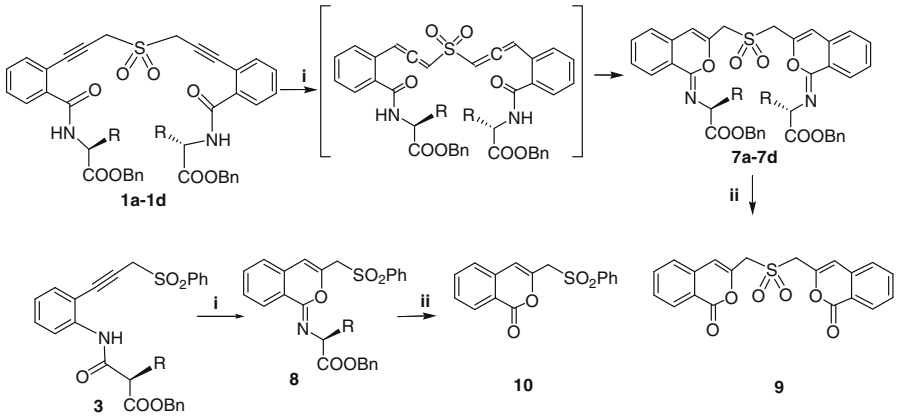
Scheme 1. Possible pathways for the bis-allenic sulfone.



a, R = Me, b, R = $CHMe_2$, c, R = CH_2CHMe_2, d, R = CH_2Ph

i = MsCl, NEt_3, 0 ^{0}C, DCM; LiBr, THF; **ii** = Na_2S, TBAB, THF-H_2O; **iii** = PhSH, NEt_3, DCM; **iv** = mCPBA, DCM

Scheme 2. Synthesis of the target bis-propargyl sulfones.



a, R = Me, **b**, R = $CHMe_2$, **c**, R = CH_2CHMe_2, **d** = CH_2Ph

i = Et_3N, $CHCl_3$, 10 min ; **ii** = HCl, MeOH, 24h

Scheme 3. Synthesis of bis-isochromen-1-imine and bis-isochromenone.

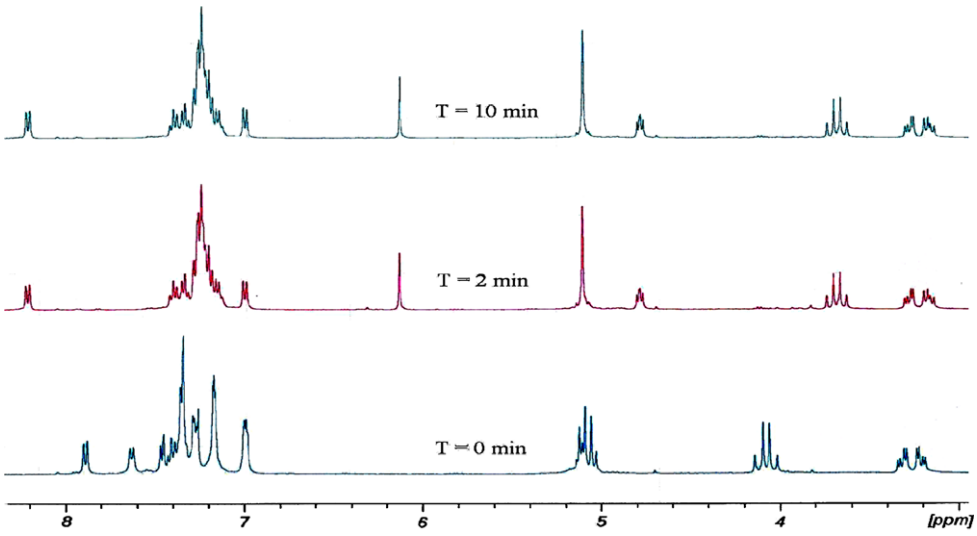


Figure 1. ^{1}H NMR at different time points upon base treatment of sulfone **1d**.

was apparent from the symmetrical nature of its structure (like appearance of only one signal for the 4,4′-positions and absence of the amide NH signal). The other alternative isoquinolone structure, however, could not be ruled out on the basis of NMR or mass spectra. The structures were finally confirmed to be the (1*H*)-isochromen-1-imine derivatives by facile acid-mediated hydrolysis to the bis-isocoumarin, which could be characterized by single crystal X-ray (the ORTEP diagram shown in Fig. 2).

The generality of this methodology for the synthesis of other bis-(1*H*)-isochromen-1-imine derivatives was demonstrated by carrying out the reaction with various other sulfones, derived from different amino acids. In each case the imine derivatives were obtained in excellent yields. The reaction also works with monopropargyl sulfones **3**. The results are shown in Table 1.

Because of the reported antiangiogenic activity of isocoumarins,[11] we became interested to check such activity of the synthesized bis-isochromene **7d**, bis-isocoumarin **9** and also the monoisochromene **8** by looking at the inhibitory activity against RNase A. Incidentally, RNase A and angiogenin have high degree of homology[12] and hence inhibition of RNase A is regarded as a model for the development of angiogenin inhibitors.[13] Thus, a solution containing RNA was incubated[14] with RNase A in the presence of various compounds and then analyzed by agarose gel electrophoresis. The results are shown in Figure 3a and b and the intensities of various bands are shown in Table 2a and b. From the tables, it is clear that although all the three compounds showed RNase inhibition, the activity shown by the bis-isochromene **7d** is significantly higher than the other compounds (Fig. 3a) and also the percentage of inhibition increased as the concentrations of **8** and **7d** were increased (Fig. 3b). It is also interesting to note that simple monoisochromene has only weak inhibition thus emphasizing the importance of a bis-system and the importance of a C_2-symmetry is being explored.

In conclusion, we have developed a simple method for the preparation of 3,3′-bis-isochromen-1-imine derivatives connected via a functional linker involving double Michael type addition to bis-allenic sulfone, generated in situ from bis-propargyl sulfone. We have also demonstrated that intramolecular nucleophilic addition

Table 1
Results of triethylamine treatment

Substrate	Product	Time (min)	Yield (%)	Yield of deprotected isocoumarins (%)
1a	**7a**	2	97	>95 (for 9) >95 (for 10)
1b	**7b**	5	95	
1c	**7c**	10	95	
1d	**7d**	2	98	
3	**8**	2	98	

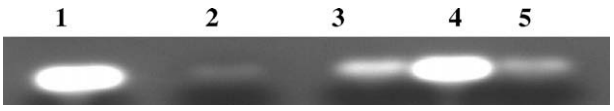


Figure 3a. RNase A inhibition study of **8**, **7d** **9**.

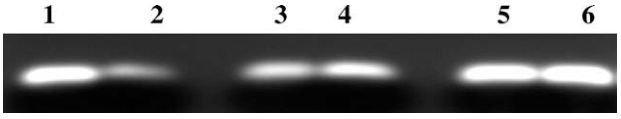


Figure 3b. RNase A inhibition study of **8** and **7d** at different concentrations.

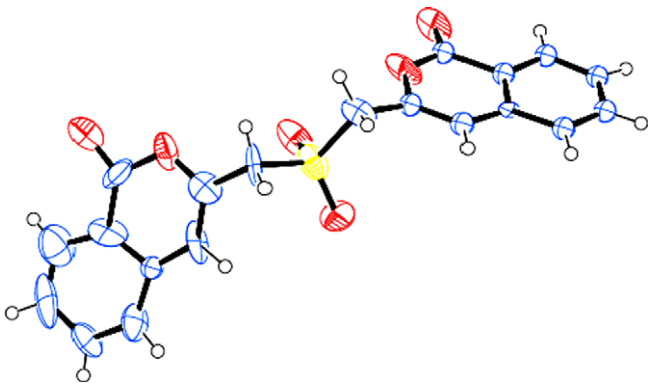


Figure 2. ORTEP diagram of bis-isocoumarin **9**.

Table 2a
RNase A inhibition studies by gel electrophoresis; compounds were taken in MeOH at 1.15 mM concentration

Lane	Content	Relative intensity
1	RNA	1
2	RNA + RNase A	0.0319
3	RNA + RNase A + **8**	0.2115
4	RNA + RNase A + **7d**	0.8985
5	RNA + RNase A + **9**	0.1144

Table 2b
RNase A inhibition studies of compounds **8** and **7d** at different concentration

Lane	Content	Relative intensity
1	RNA	1
2	RNA + RNase A	0.3689
3	RNA + RNase A + **8** (1 mg/mL)	0.5800
4	RNA + RNase A + **8** (2 mg/mL)	0.6173
5	RNA + RNase A + **7d** (1 mg/mL)	0.9720
6	RNA + RNase A + **7d** (2 mg/mL)	0.9915

of amides is more facile than the Garratt–Braverman cyclization pathway which takes place at room temperature in aryl-substituted allenes. Our observation is similar to our recently reported synthesis of bis-indoles involving nucleophilic addition of amide nitrogen to allenic sulfones[15] as well as cleavage of DNA via alkylation pathway.[16] The newly synthesized bis-isochromenes and the biscoumarin possess strong RNase A inhibition activity thus demonstrating the potential importance of these compounds.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.03.075.

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14. *Incubation condition:* The incubation was done at rt in MeOH for 2 h. For Figure 3a, a 1.15 mM stock solution of each of **7d**, **8** and **9** was used. For Figure 3b, the concentrations of the compounds **7d** and **8** are the concentrations of the stock (effective concentrations are three times less). The stock concentrations of the RNA and RNase A are 10 mg/mL and 0.8 μM, respectively.
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Spectral data of selected compounds: All the ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, in $CDCl_3$. Specific rotation was measured in $CHCl_3$.
For **7a**: δ_H 8.26 (d, *J* = 7.2 Hz, 2H), 7.51–7.14 (m, 16H), 6.28 (s, 2H), 5.23–5.09 (m, 4H), 4.69 (app. q, *J* = 7.0 Hz, 2H), 3.98 (d, *J* = 14.4 Hz, 2H), 3.79 (d, *J* = 14.4 Hz, 2H), 1.56 (d, *J* = 7.0 Hz, 6H); δ_C 175.1, 155.3, 135.9, 128.8, 127.1, 125.6, 123.4, 121.5, 115.6, 114.1, 80.2, 53.1, 51.7, 28.3, 18.6; $[\alpha]_D^{25}$ −44.5 (*c* 0.25); MS: *m*/*z* = 727.34 [MNa$^+$], 705.33 [MH$^+$]; HRMS: calcd for $C_{40}H_{36}N_2O_8S + H^+$ 705.2272 found 705.2275.
For **7d**: δ_H 8.21 (d, *J* = 7.6 Hz, 2H), 7.42–7.13 (m, 24H), 7.00 (d, *J* = 7.6 Hz, 2H), 6.14 (s, 2H), 5.11 (s, 4H), 4.79 (dd, *J* = 8.0, 6.0 Hz, 2H), 3.69 (dd, *J* = 29.8, 14.8 Hz, 4H), 3.28 (dd, *J* = 13.2, 5.6 Hz, 2H), 3.17 (dd, *J* = 13.2, 8.0 Hz, 2H); δ_C 172.0, 142.3, 138.3, 135.8, 132.2, 131.4, 129.6, 129.1, 128.4, 128.3, 128.1, 127.3, 126.4, 125.4, 108.8, 66.5, 60.8, 57.0, 39.9; $[\alpha]_D^{25}$ −49.9 (*c* 0.25); MS: *m*/*z* = 879.22 [MNa$^+$], 857.25 [MH$^+$]; HRMS: calcd for $C_{52}H_{44}N_2O_8S + H^+$ 857.2899 found 857.2888.
For **8**: δ_H 8.25 (d, *J* = 5.6 Hz, 1H), 7.72 (d, *J* = 7.2 Hz, 2H), 7.55–7.13 (m, 16H), 6.08 (s, 1H), 5.11 (dd, *J* = 22.8, 12.4 Hz, 2H), 3.96–3.93 (m, 1H), 3.71 (dd, *J* = 24.0, 14.4 Hz, 2H), 3.13–3.08 (m, 1H), 3.01–2.96 (m, 1H); δ_C 172.8, 143.1, 138.3, 138.1, 135.9, 134.1, 132.3, 131.7, 129.5, 129.4, 129.2, 129.0, 128.5, 128.4, 128.3, 128.0, 127.4, 126.4, 125.4, 116.1, 1.8.6, 66.4, 60.2, 60.1, 39.6; $[\alpha]_D^{28}$ −31.4 (*c* 0.25); MS: *m*/*z* = 560.06 [MNa$^+$], 538.08 [MH$^+$]; HRMS: calcd for $C_{32}H_{27}NO_5S + H^+$ 538.1689 found 538.1685.
For **9**: δ_H 8.29 (d, *J* = 8.0 Hz, 2H), 7.76 (t, *J* = 7.6 Hz, 2H), 7.58 (t, *J* = 7.6 Hz, 2H), 7.48 (d, *J* = 8.0 Hz, 2H), 6.77 (s, 2H), 4.37 (s, 4H); δ_C 161.2, 144.2, 135.9, 135.2, 129.8, 128.5, 126.2, 120.8, 110.2, 57.8; MS: *m*/*z* = 405.28 [MNa$^+$], 383.28 [MH$^+$]; HRMS: calcd for $C_{20}H_{14}O_6S + H^+$ 383.0590 found 383.0596.