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Syntheses of Imidazole-Acridine Conjugates as Ribonuclease A Mimics

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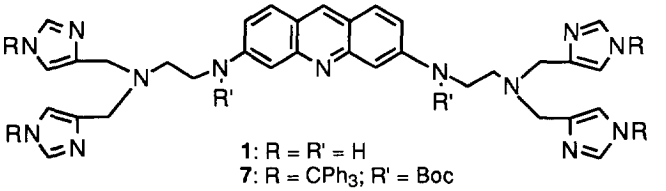
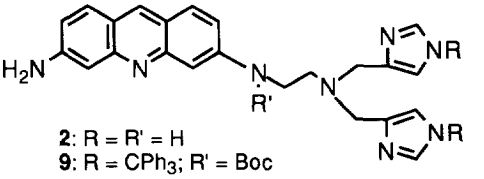
Abstract: *As a result of efforts to mimic the activity of RNase A we report the syntheses of two novel molecules **1** and **2** containing imidazole residues conjugated to an acridine, a well known intercalator and as such these molecules simultaneously have substrate recognition and potential catalytic ability. t-RNA cleavage by **1** and **2** is reported.* Copyright © 1996 Elsevier Science Ltd

In recent years RNA cleavage agents have received considerable attention partly because of their possible therapeutic application. However, the development of a small synthetic organic molecule which mimics naturally occuring ribonuclease with efficient RNA cleaving activity has not been successful yet. Typically, compounds with RNAase activity have been metal complexes,[1] oligoamines[2] and polypeptides.[3] A hybrid of ethylenediamine and a DNA oligomer selectively hydrolyses linear RNA.[4] In a different approach molecules which imitate the active centers of ribonucleases have been synthesized as models with potential to achieve very high enzymatic hydrolytic rates. Hence RNase A which is structurally well known[5] and has two catalytic groups (the imidazole rings of histidine-12 and histidine-119) has been mimicked. Breslow reported[6] the cleavage of phosphodiester bonds of some model substrates with β-cyclodextrin derivatives carrying two imidazole groups. Moreover, molecules containing an intercalating dye such as those found in the peptide-acridine conjugate series[7] or those containing imidazole residues conjugated to an acridine[8] or to a phenazine[9] derivative by linkers of variable length and flexibility display hydrolytic activity. Resulting from our efforts to increase the effectiveness of these molecules in RNA hydrolysis we describe the synthesis of compounds **1** and **2** containing imidazole rings conjugated to an acridine unit.



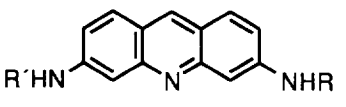
1: R = R' = H
7: R = CPh_3; R' = Boc



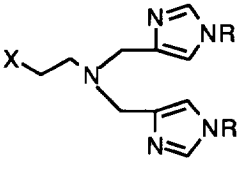
2: R = R' = H
9: R = CPh_3; R' = Boc

Our strategy in the synthesis of these compounds was to develop a molecule in which the substrate binding capability of RNase A could be mimicked by an intercalator used as a vehicle for carrying imidazole residues close to the phosphodiester bonds to be hydrolyzed.

Synthesis of the compound 1. For the synthesis of **1** we planned to use 3,6-diaminoacridine (proflavine) as the starting material. In the first step the exocyclic amino groups were protected as *tert*-butyl carbamates. By heating a solution of proflavine (2 g) in dry acetone (250 ml) at reflux for 72 h with an excess (12 eq) of di-*tert*-butyl pyrocarbonate, afforded **3** (72% yield) and **8** (10% yield) as a by-product of the reaction. As starting material for the non-intercalating moiety, 4(5)-hydroxymethylimidazole hydrochloride was prepared from fructose by the Darby and Totter procedure.[10] The treatment of this compound with thionyl chloride in benzene according to the described method[11] yields 4(5)-chloromethylimidazole which was then heated at reflux for 3.5 h with 2-aminoethanol (0.5 eq) and triethylamine (2 eq). Work-up of the reaction mixture (evaporation of the solvent, addition of dichloromethane, isolation of the resulting oil and flash column chromatography on silica gel with 50% methanol-ethyl acetate as the eluent) afforded pure *N,N*-bis(4(5)-imidazolylmethyl)aminoethanol (**4**) in 56% yield. By stirring a solution of **4** in DMF at room temperature for 24 h with an excess of thionyl chloride, 2-chloro-*N,N*-bis(4(5)-imidazolylmethyl)ethylamine trihydrochloride (**5**) was obtained in 86% yield.



3: R = R' =Boc
8: R = H; R' = Boc



4: X = OH; R = H
5: X = Cl; R = H
6: X = Cl; R = CPh_3

The next step of the synthetic procedure involved the reaction of dicarbamate **3** with the trihydrochloride **5**. However, all attemps to perform this reaction under different conditions were unsuccessful. Consequently, protection of the imidazole nitrogen seemed necessary. We chose the triphenylmethyl group because: a) it is sufficiently robust to allow subsequent synthetic manipulations under basic conditions, b) it is easily removed by mild acid hydrolysis and c) the protection occurs regioselectively affording only the 4-substituted product **6** presumably because of its steric bulk.[12] Protection was performed by addition of a solution of triphenylmethylchloride (2 eq) in dry DMF to a stirred mixture of the trihydrochloride **5** and triethylamine (5.5 eq) in dry DMF. Work-up of the reaction mixture afforded pure **6** in 49% yield. The reaction of the protected compound with dicarbamate **3** was successfully performed according to the following procedure: a mixture of **3** (84 mg, 0.21 mmol) , 80% NaH (18.5 mg, 0.62 mmol) and **6** (327 mg, 0.45 mmol) in dry DMF (10 ml) was stirred at room temperature under an argon atmosphere for 24 h and then poured onto crushed ice. The precipitate was collected, washed with water, dried and purified by column chromatography on silica gel using 5% triethylamine-ethyl acetate as eluent, yielding pure **7**[13] (197 mg, 54 %). A small amount of mono-alkylated compound was also recovered from the column. Finally, all the protecting groups (*tert*-butylcarbamate and triphenylmethyl) were easily removed by treating **7** at 60° for 2 h with 2N hydrochloric acid. Filtration of the precipitate and basification (pH = 9) of the filtrate afforded the RNase mimic **1**[14] in 87% yield.

Synthesis of the compound 2. The first step in the synthesis of **2** involved preparation of the monocarbamate **8**. By heating at reflux a solution of proflavine (2 g) in dry acetone (250 ml) for 8 h with an excess of di-*tert*-butyl pyrocarbonate (5 eq), afforded monocarbamate **8** (40% yield) and dicarbamate **3** (7% yield). The reaction of the monocarbamate with the chloride **6** afforded **9** in 55% yield. The reaction was performed according to the following procedure: To a solution of **8** (155 mg, 0.5 mmol) in dry DMF, 80% NaH (30 mg, 1 mmol) was added. The reaction mixture was stirred at room temperature under argon for 15 min and then **6** (400 mg, 0.55 mmol) was added. The mixture was maintained with stirring at room temperature for 48 h with one further portion of **6** (120 mg, 0.16 mmol) being added during this period. Work-up (pouring onto crushed ice, dissolving the precipitate in dichloromethane, washing twice with water, drying of the organic phase and flash column chromatography on silica gel using ethyl acetate-acetone-triethylamine (5/5/1, v/v/v) as eluent) afforded **9**.[15] All the protecting groups were removed with 2N hydrochloric acid at 60° for 2h. Filtration of the precipitate and basification (pH = 9) of the filtrate afforded **2**[16] in 85% yield.

RNA Cleavage Reaction

Reaction mixtures contained 5'- or 3'-end labelled RNAs (50-100 000 cpm) supplemented with 1.5 μg of carrier t-RNA and concentrations of compounds **1** and **2** (100 μM) dissolved in 20 μl of reaction buffer (20 mM HEPES, pH 7.0, 100 mM NaCl, 10 mM $MgCl_2$, 1mM EDTA). The RNA-complexes precipitated with concentrations of compounds above 100 μM. After incubation, RNAs were precipitated with ethanol. The reaction times were 4, 12 and 24 hours. The precipitated RNA products were recovered by centrifugation and the precipitate was washed with 70% ethanol and dried, and analysed for cleavage products. Samples were dissolved in 7 M urea containing 0.1% bromophenol blue and xylene cyanole and subjected to electrophoresis through a 10% polyacrylamide sequencing gel.

Cleavage of t-RNA by compounds **1** and **2** was obtained whereas no detectable cuts were generated by compound **4** after 24 hours. Compound **1** was shown to have a high catalytic activity, since after 4 hours most of the t-RNA had been cleaved.

These preliminary results have encouraged us to continue working with these synthetic products and to explore the specificity, sensitivity and the mechanism of the cleavage reaction.

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13. Compound **7**: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.39 (s, 18H, *t*-But), 2.84 (t, 4H, J= 7.32 Hz, CH_2N), 3.58 (s, 8H, $C\underline{H}_2Im$), 3.92 (t, 4H, J= 7.32 Hz, $C\underline{H}_2N$-acridine), 6.49 (d, 4H, J= 1.1 Hz, H-5 imidazole), 7.01-7.26 (m, 60 H, Ph-H), 7.31 (d, 4H, J= 1.1 Hz, H-2 imidazole), 7.43 (dd, 2H, J= 9.15 Hz, J'= 1.83 Hz, H-2(7) acridine), 7.62 (d, 2H, J= 9.15 Hz, H-1(8) acridine), 7.87 (d, 2H, J= 1.83 Hz, H-4(5) acridine), 8.37 (s, 1H, H-9 acridine), MS (FAB) *m/e* 1785 (M^++H) (calcd $C_{119}H_{109}N_{13}O_4$ 1783.8)
14. Compound **1**: ^{1}H-NMR (CD_3OD, 300 MHz) δ (ppm): 2.76 (t, 4H, J= 5.86 Hz, CH_2N), 3.28 (t, 4H, J= 5.86 Hz, $C\underline{H}_2N$-acridine), 3.69 (s, 8H, $C\underline{H}_2Im$), 6.65 (d, 2H, J= 2.20 Hz, H-4(5) acridine), 6.90 (dd, 2H, J= 9.15 Hz, J'= 2.20 Hz, H-2(7) acridine), 6.97 (d, 4H, J= 0.73 Hz, H-5 imidazole), 7.58-7.62 (m, 6H, H-1(8) acridine and H-2 imidazole), 8.30 (s, 1H, H-9 acridine), MS (FAB) *m/e* 616 (M^++H) (calcd $C_{33}H_{37}N_{13}$ 615.3).
15. Compound **9**: ^{1}H-NMR (CD_3OD, 300 MHz) δ (ppm): 1.37 (s, 9H, *t*-But), 2.74 (t, 2H, J= 7.30 Hz, CH_2N), 3.52 (s, 4H, $C\underline{H}_2Im$), 3.82 (t, 2H, J= 7.30 Hz, $C\underline{H}_2N$-acridine), 6.54 (s, 2H, H-5 imidazole), 6.94-7.38 (m, 35H, 3H acridine, 2H imidazole and 30H Ph-H), 7.62 (d, 1H, J= 8.79 Hz, H-8 acridine), 7.72 (d, 1H, J= 1.1 Hz, H-4 acridine), 7.80 (d, 1H, J= 9.16 Hz, H-1 acridine), 8.49 (s, 1H, H-9 acridine).
16. Compound **2**: ^{1}H-NMR (CD_3OD, 300 MHz) δ (ppm): 2.77 (t, 2H, J= 6.59 Hz, CH_2N), 3.29 (m, 2H, $C\underline{H}_2N$-acridine), 3.69 (s, 4H, $C\underline{H}_2Im$), 6.63 (br s, 1H, H-4 or H-5 acridine), 6.88-6.95 (m, 3H, H-5 or H-4 , H-2 and H-7 acridine,), 6.97 (s, 2H, H-5 imidazole), 7.25 (t, 1H, NH-acridine), 7.62 (s, 2H, H-2 imidazole), 7.63-7.68 (m, 2H, H-1 and H-8 acridine), 8.37 (s, 1H, H-9 acridine), MS (FAB) *m/e* 413 (M^++H) (calcd $C_{23}H_{24}N_8$ 412.2).

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