The Preparation of Intercalating Isoxazoles *via* a Nitrile Oxide Cycloaddition

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Pyridinium chlorochromate oxidation of 9-methylacridine (**2**) affords the corresponding aldehyde **3** in good yield. Conversion of the aldehyde to the hydroximinoyl chloride **5a** was accomplished *via* reaction of the corresponding oxime with *N*-chlorosuccinimide. Dipolar addition to the enamine of ethylacetoacetate provided the corresponding isoxazoles **1** in good yield.

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Introduction.

Researchers have been interested in targeting AIDS at the most vulnerable stages of the HIV replication cycle. The possible targets for antiretroviral therapy can be found at each stage of the virus' life cycle. The more readily apparent targets include the gp120 binding protein [1,2,3], reverse transcriptase [4], integrase [5], and the viral-coded protease [6]. All of these targets are necessary for the life cycle of HIV, with the majority of research centered on reverse transcriptase [4]. However, these targets can exist in healthy cells, require large local concentrations of a potential drug to be effective, and/or presume that only one such target exists per host cell.

Targeting a more vulnerable stage of the replicative cycle has been the goal of this laboratory. Specifically, design of a drug to bind to a sequence within the infectious virus' DNA unique to the virus has been of interest. Such a target would circumvent the inherent problems associated with the current retroviral therapies. Similar work has been attempted with oligonucleotides [7], but due to the problems inherent with oligonucleotide therapy, relatively few therapeutic advances have been made in this area.

Many biologically active naturally occurring and synthetic molecules that are capable of interaction with B-DNA can be found in the literature [8]. Of particular interest to our laboratory are those compounds that bind to DNA by intercalation (for example, actinomycin, triostin A, and echinomycin) and those compounds which bind to DNA purely by association with the minor groove (for example, netropsin and distamycin). Netropsin and distamycin are examples of polyamidopyrroles that are specific for binding interactions with poly-AT DNA [8].

The combination of intercalators and minor-groove binders of DNA into a single bifunctional mixed ligand has been accomplished previously with promising results. Reiss and coworkers [9] have shown that acridines linked to netropsin or distamycin analogues possess greater binding affinity (ten to 1000 times greater) for DNA than either 9-aminoacridine or the minor-groove binders. Reiss' design of these bifunctional DNA ligands indicated that the optimum linker length between the acridine and the minor-groove binders would consist of a chain of 5 atoms. Their study, however, was limited to the use of flexible straight chain linkers.

As noted above, various studies have indicated that similar polyamidopyrroles exhibit a preference for binding to poly-AT DNA. Within HIV's *tat* gene are many poly-AT stretches of DNA [10], of exceptional length are the sequences between base pairs 5480-5496 (AATTGCTATTGTAAAAA), 5521-5534 (TTTCATAACAAAA), and 8651-8664 (TTTTTAAAAGAAAA). The sequence from 5480-5496 codes for a region entirely within the cysteine rich domain of the Tat protein product. This domain is highly conserved between HIV-1, HIV-2, and SIV Tat proteins [11], and its length indicates that it should be statistically unique to HIV's *tat* gene. Thus, a molecule that binds exclusively to this region would be able to exhibit its cytotoxicity with a variety of HIV strains with limited risk of the development of resistant mutants.

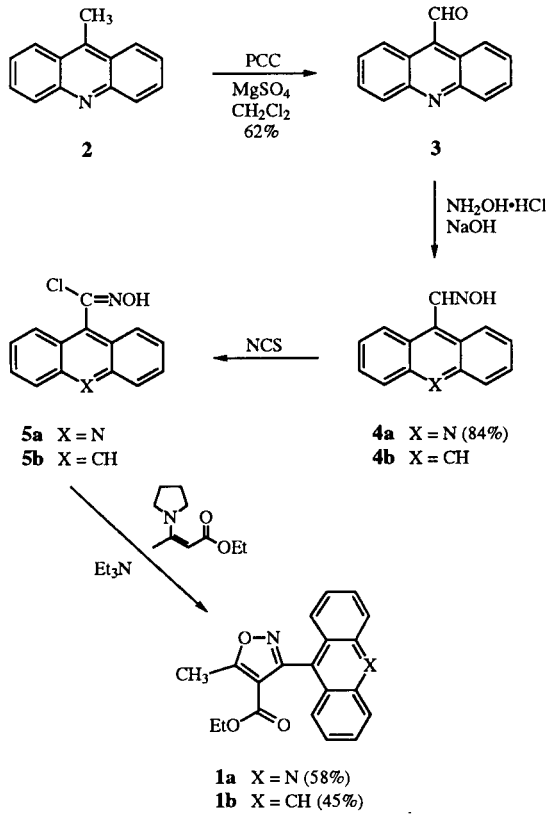
The target intercalating lexitropsin can be prepared by coupling a distamycin or netropsin analog with an isoxazole-intercalator. The use of isoxazoles as the linking agent between a distamycin-like polyamidopyrrole will serve three purposes. First, the attachment can be easily accomplished by condensation of an amine on the polyamidopyrrole and a 3-intercalator-5-methyl-4-acyl chloride isoxazole. The isoxazole acid chloride can be prepared from the acid using standard methodology [12].

Second, the isoxazole will serve as a prodrug for the delivery of an intercalating lexitropsin to the cellular DNA [13]. Finally, the distance required for the optimum tether length, as indicated by Reiss, between the intercalator and the minor-groove binder would be 5 atoms. However, in this case, the tether would exhibit restrictive conformations.

Synthesis of the isoxazole-intercalators **1a** and **1b** has been accomplished in this laboratory as indicated in the Scheme. The compounds were prepared *via* an interesting 1,3-dipolar cycloaddition involving the hydroximinoyl chlorides **5** and the enamine of ethyl acetoacetate. The hydroximinoyl chlorides **5** were prepared from the oximes as indicated using *N*-chlorosuccinimide.

Preparation of 9-methylacridine (**2**) was accomplished

Scheme: Isoxazole-Intercalator Preparation



using the procedure of Bernthsen [14]. The resulting compound was isolated by column chromatography and agreed with the spectroscopic data reported for this compound [15].

Oxidation of 9-methylacridine (**2**) to 9-acridinaldehyde (**3**) has been previously accomplished by Monti by heating with selenium(IV) oxide for 2-3 hours on a sand bath [16]. However, we have found it convenient to prepare the aldehyde **3** by pyridinium chlorochromate oxidation of 9-methylacridine (**2**). This method involved addition of the methylacridine to 1.05 molar equivalents of pyridinium chlorochromate vigorously stirred in dichloromethane at room temperature. Magnesium sulfate was added to act as a suspension matrix for the reduced chromium. The resulting black-brown slurry was quickly filtered to give the desired product in 62% yield after chromatography. Similar benzylic oxidations with pyridinium chlorochromate have been reported [17], but usually require harsher conditions and large excesses of pyridinium chlorochromate as an oxidant.

Proof of the structure of 9-acridinaldehyde was obtained by converting **3** into its corresponding oxime **4a** [16]. Compound **4b**, 5-anthracenecarboxaldehyde oxime, was commercially available. The oximes were then chlorinated using the procedure of Liu and Howe [18]. It was discovered that the hydroximinoyl chlorides **5a** and **5b** were formed quite rapidly without the use of hydrogen chloride gas as a catalyst. This stands in contrast to the preparation of substituted benzohydroximinoyl chlorides which require addition of small amounts of hydrogen chloride to act as an initiator of the reaction [18].

The hydroximinoyl chlorides **5a** and **5b** were then added to a solution of the enamine of ethyl acetoacetate and triethylamine to provide the isoxazoles [19]. The mechanism of this reaction involves the formation of a nitrile oxide which undergoes a 1,3-dipolar cycloaddition to the enamine. After elimination of pyrrolidine, the isoxazole can be easily isolated by extractive isolation and purified by chromatography.

Close examination of the ^{1}H nmr of the isoxazole esters **1a** and **1b** revealed an upfield shift for each of the ethyl ester protons. This shift is approximately -1.0 ppm for the methyl protons and -0.4 ppm for the methylene protons when compared to the chemical shift of an aliphatic ester (such as ethyl acetate). It is postulated that the shift arises from the magnetic anisotropy associated with the ring system attached to the isoxazole C(3) position, indicating that the isoxazole and the polycyclic aromatic rings are perpendicular to each other and the ethyl ester lies underneath the polycyclic aromatic rings and within the field associated with the ring system.

EXPERIMENTAL

Mass spectra were obtained on a VG 7070 gc/ms with a model 11/250 data system. The nmr spectra (^{1}H and ^{13}C) were obtained on a Brüker AF200 multinuclear FT-NMR (200 MHz for ^{1}H). Combustion analyses were performed by Desert Analytics Laboratory, PO Box 41838, Tucson, Arizona. All reactions were performed under an inert atmosphere of nitrogen or argon. Tetrahydrofuran was distilled from sodium-benzophenone ketyl immediately before use. Flash chromatography was performed on silica gel (70-230 mesh) with freshly distilled solvents by the method of Still [20]. The enamine of ethyl acetoacetate was prepared by the method of McMurry and stored as a neat oil until needed [19,21]. The method of Bernthsen [14] was used to prepare 9-methylacridine.

9-Acridinaldehyde (**3**).

To a vigorously stirred slurry of pyridinium chlorochromate (2.0 g, 9.2 mmoles) and magnesium sulfate (5.0 g) in dichloromethane (50 ml) was added 9-methylacridine (1.7 g, 8.8 mmoles). The resulting mixture was stirred at room temperature overnight, then diluted with 200 ml of diethyl ether. The brown slurry was filtered with suction through a small pad of silica gel and the filtrate dried over magnesium sulfate, filtered, and evaporated to afford the crude product as a slightly red oil. Flash chromatography (hexane:ethyl acetate 9:1) gave 1.15 g (62%) of the desired product as a yellow solid that agreed with the literature data for 9-acridinaldehyde [16]; ^{1}H nmr (deuteriochloroform): δ 11.51 (s, 1H, C*H*O), 8.71 (d, J = 8.2 Hz, 2H, acridine), 8.26 (d, J = 8.7

Hz, 2H, acridine), 7.79 (q, J = 7.8 Hz, 2H, acridine), 7.68 (q, J = 7.0 Hz, 2H, acridine); ir (nujol): ν CO 1680 cm^{-1}.

9-Acridinaldehyde Oxime (**4a**) Hydrochloride.

To a solution of **3** (1.15 g, 5.5 mmoles) in 95% ethanol (20 ml) was added water (2 ml), ice (5 g), and hydroxylamine hydrochloride (0.50 g). The resulting mixture was stirred at 0° for 15 minutes, then, 2 ml of aqueous sodium hydroxide (2.0 molar) was added dropwise with stirring causing precipitation of an orange solid. After 3 hours at room temperature, the reaction was made basic (*p*H 9) and extracted with ether (2 x 50 ml) to remove unreacted starting material (0.80 g, 70% recovery). The aqueous phase was acidified to *p*H 6 with concentrated hydrochloric acid and extracted with chloroform (3 x 50 ml). The chloroform extracts were dried over magnesium sulfate, filtered, and evaporated to give 0.35 g (26%, 84% based on recovered starting material) of the desired product as a yellow solid that agreed with literature values; ^{1}H nmr (perdeuteriodimethyl sulfoxide): δ 12.27 (s, 1H, *H*Cl), 10.16 (s, 1H, O*H*), 9.28 (s, 1H, *H*C=N), 8.53 (d, J = 8.7 Hz, 2H, acridine), 8.18 (d, J = 8.7 Hz, 2H, acridine), 7.86 (dd, J = 8.7, 8.7 Hz, 2H, acridine), 7.65 (dd, J = 8.7, 8.7 Hz, 2H, acridine); ^{13}C nmr (perdeuteriodimethyl sulfoxide): δ 144.9, 130.4, 129.3, 126.7, 126.2, 126.0, 125.6, 123.5.

Hydroximinoyl Chlorides **5a** and **5b**.

The oxime **4a** or **4b** (1.25 mmoles) was dissolved in freshly distilled dimethylformamide (10 ml) and at room temperature *N*-chlorosuccinimide (1.15 mmoles) was added portionwise with stirring. The solution became warm after the first portion of *N*-chlorosuccinimide was added. After complete addition, the mixture was stirred at room temperature overnight. The reaction was partitioned between water (50 ml) and ether (50 ml) and the aqueous phase extracted with ether (2 x 50 ml). The combined ethereal phases were washed with water (3 x 25 ml), dried over magnesium sulfate, filtered, and evaporated to provide the desired crude products. These compounds were used immediately without further purification.

General Procedure for the Preparation of **1a** and **1b**.

A solution of the crude hydroximinoyl chloride (1.14 mmoles) in absolute ethanol (25 ml) was added to the enamine of ethyl acetoacetate (0.30 g, 1.64 mmoles) and triethylamine (1 ml) in absolute ethanol (15 ml). The resulting red solution was stirred at room temperature overnight. It was then dissolved in 50 ml of hydrochloric acid (1.0 molar) and extracted with ether (3 x 50 ml). The combined ethereal phases were washed with water (1 x 20 ml), dried over magnesium sulfate, filtered, and evaporated to give the crude products.

Ethyl 3-(9'-Acridinyl)-5-methyl-4-isoxazolecarboxylate (**1a**).

Flash chromatography (hexane to hexane:acetone 8:2 gradient elution) of the crude oil provided the desired product (58% yield from **4a**) as a yellow solid; ^{1}H nmr (deuteriochloroform): δ 8.27 (d, J = 8.8 Hz, 2H, acridine), 7.75 (ddd, J = 1.3, 8.7, 8.8 Hz, 2H, acridine), 7.64 (d, J = 8.7 Hz, 2H, acridine), 7.45 (ddd, J = 1.3, 8.7, 8.8 Hz, 2H, acridine), 3.66 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 2.88 (s, 3H, CH_3), 0.29 (t, J = 7.1 Hz, 3H, OCH_2CH_3); ^{13}C nmr (deuteriochloroform): δ 176.5, 161.0, 157.8, 148.3, 133.4, 130.2, 129.6, 126.5, 125.5, 125.2, 111.2, 60.3, 13.3, 12.8; ms: (fast-atom-bombardment) m/z 333 (M+1), 287 (M+-OCH_2CH_3), 245, 205 (9-cyanoacridine 1+), 179 (acridine 1+), 154; ms: (fast-atom-bombardment, exact-mass): Calcd. for $C_{20}H_{17}O_3N_2$: 333.1239. Found: 333.1258.

Anal. Calcd. for $C_{20}H_{16}O_3N_2 \cdot 0.5H_2O$: C, 70.37; H, 5.02; N, 8.20. Found: C, 70.46; H, 5.06; N, 7.99.

Ethyl 3-(5'-Anthracenyl)-5-methyl-4-isoxazolecarboxylate (**1b**).

Flash chromatography (hexane to hexane:acetone 8:2 gradient elution) of the crude oil provided the desired product (45% yield from **4b**) as a yellow solid; ^{1}H nmr (deuteriochloroform): δ 8.55 (s, 1H, anthracene), 8.02 (dd, J = 2.2, 6.4 Hz, 2H, anthracene), 7.64 (dd, J = 2.2, 6.4 Hz, 2H, anthracene), 7.40 (m, 4H, anthracene), 3.68 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 2.89 (s, 3H), 0.29 (t, J = 7.1 Hz, 3H, OCH_2CH_3); ^{13}C nmr (deuteriochloroform): δ 176.5, 161.5, 160.4, 138.1, 131.0, 130.8, 128.6, 128.4, 126.3, 125.4, 125.2, 120.6, 60.0, 13.4, 12.8.

Anal. Calcd. for $C_{21}H_{17}O_3N$: C, 76.12; H, 5.17; N, 4.23. Found: C, 76.31; H, 5.01; N, 4.10.

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