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Preparation of alkylation agents for bulged DNA microenvironments

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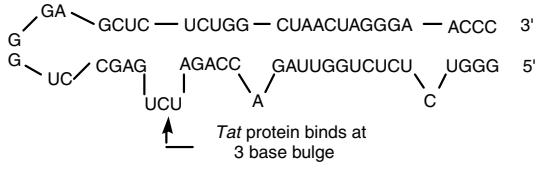
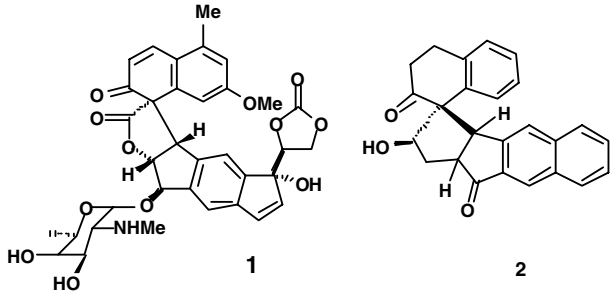
Abstract—A designed molecule with capacity to alkylate DNA bulges has been prepared from readily available starting materials. The spirocyclic template utilized was designed on the basis of established architectures, and equipped with a mustard alkylating group. Preliminary studies confirm alkylation of specific bulged sequences, paving the way for second generation substrates with higher affinity.

Nucleic acids can have richly diverse structures, including hairpins, knots, pseudoknots, triple helices, loops, helical junctions, and bulges.[1] Such bulged structures in nucleic acids are of general biological significance,[2] having been proposed as intermediates in processes as diverse as RNA splicing, frame-shift mutagenesis, intercalator induced mutagenesis, and imperfect homologous recombination.[3] Bulges have also been suggested as *binding motifs* for regulatory proteins involved with viral replication, including the TAR region of HIV-1,[4–6] and have been implicated in the etiology of a number of human neurodegenerative genetic diseases.[7] Though compounds capable of binding to bulges could have therapeutic potential, progress has been limited. Our interest in this problem was stimulated when it was demonstrated that a metabolite of the naturally occurring enediyne NCS, specifically arene **1** (R = cyclic carbonate) had selective affinity for bulged sequences.[8] On the basis of molecular modeling and 2D NMR data it became apparent that the unprecedented spirolactone moiety conferred affinity, by virtue of presenting the two arene units in such a manner as to mimic the base paring at the bulged site. Spurred by this finding, we initiated a program to define the minimal structural subunit that might recognize the bulged environment and that could be attained through chemical synthesis. Sprioalcohol **2**, available via intramolecular aldol chemistry became the lead compound, showing micromolar affinity for 2 base bulges, and which could be increased significantly by subsequent aminoglycosylation.[9]



TAR RNA region of HIV-1



With affinity for bulged sequences demonstrated, we now wished to show irreversible bulge binding via an

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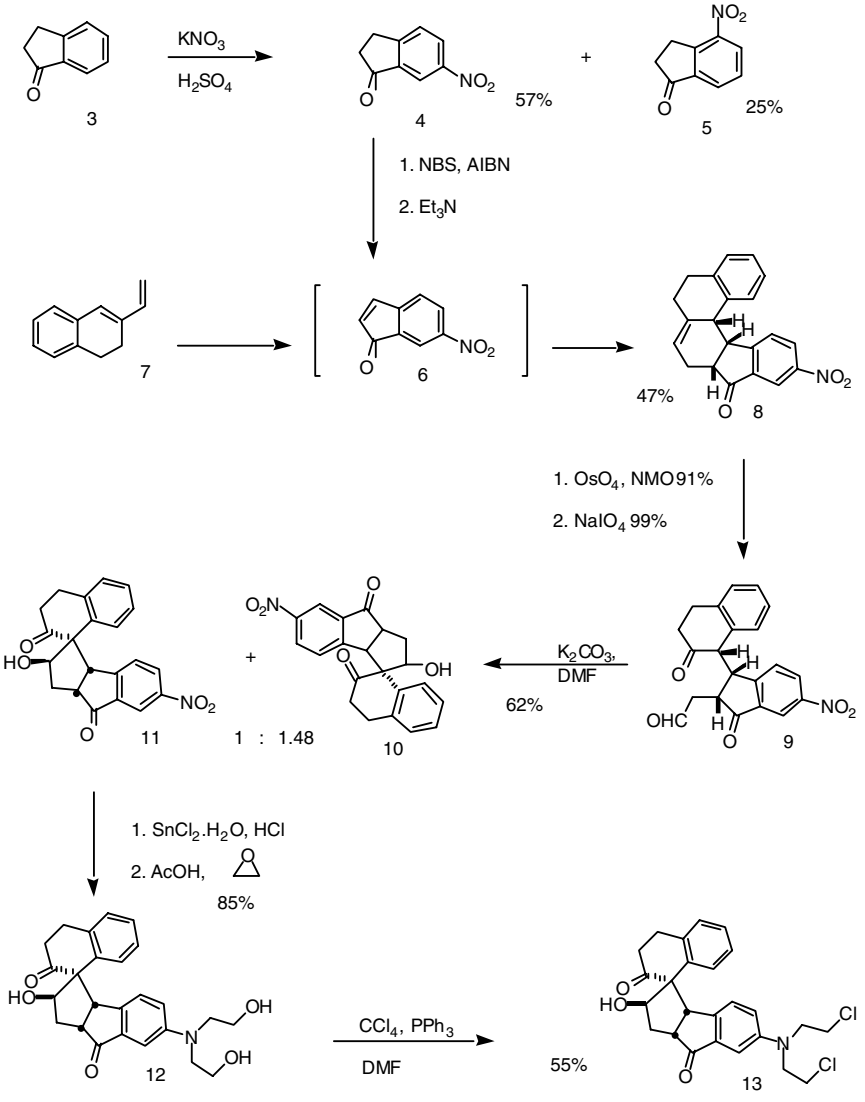
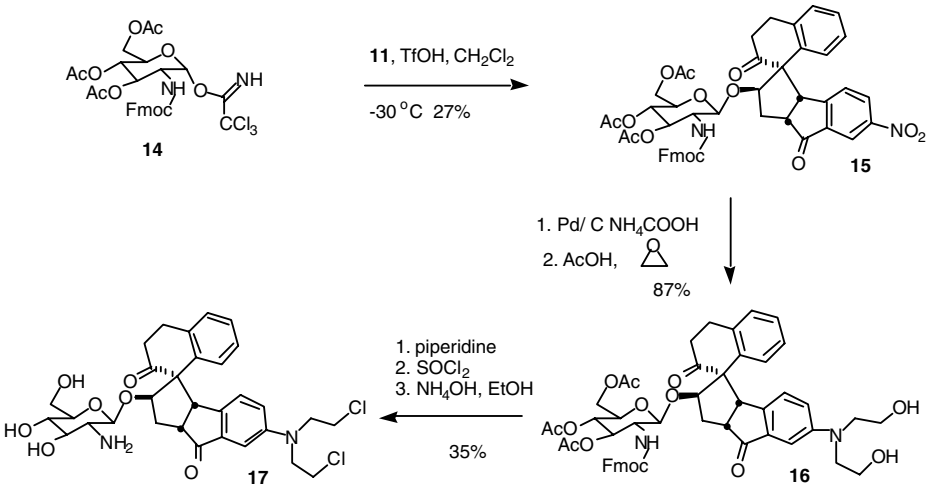
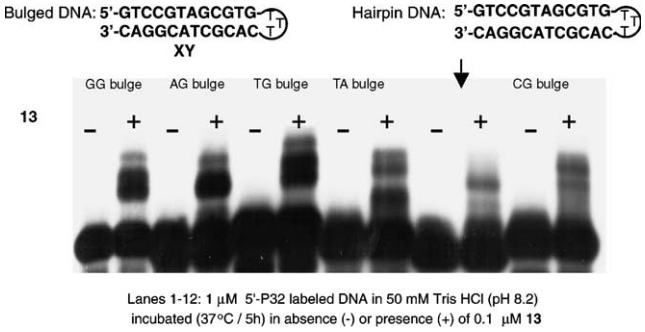
alkylating derivative. Such derivatives, in addition to giving insight to the binding pockets of bulged microenvironments, could potentially be useful as molecular therapeutics. Based on prior experience with intercalator mustard agents[10] we set about synthesis of β-chloroethylamine derivatives of the spirocycle template. Commercially available indanone **3** was subjected to standard nitration, yielding a mix of **4** and **5** in favor of the desired compound (Scheme 1).[11] Bromination α to the ketone followed by concomitant elimination allowed cycloaddition of dienophile **6**, with diene **7**, in turn prepared from acetyl tetralone. The resulting cycloaddition product **8** was obtained in moderate yield, and then subjected to classic oxidative cleavage to produce keto aldehyde **9**. The critical aldol reaction proceeded as planned, giving desired spiroalcohol **11** and the *exo*-isomer **10**, which could be separated easily via silica gel chromatography. With the spiro junction intact and the progenitor of the arylamine in place, we attempted introduction of the chloro mustard group without protection of the spiroalcohol group. Thus nitro reduction followed by reaction with ethylene oxide gave diol **12**, which underwent smooth and selective primary chlorination to yield mustard agent **13** in good yield. With a substrate in hand, screening for alkylation ability against a small panel of bulged substrates was conducted. Preliminary assays confirm bulge alkylation, with some discriminatory preference observed for specific sequences (lanes 2,4,6 cf. lanes 8, 12) versus a control duplex hairpin (lane 10). Though alkylation efficiency is moderate (max ~15%, lane 2) these findings are encouraging, particularly in light of the fact that pendant aminoglycosyl functionality confers substantial additional affinity and selectivity to bulged binding.[9,12] To demonstrate compatibility of the glycosylation chemistry with the current protocol, an aminoglucose derivative was synthesized (Scheme 2). Spirocycle **11** was coupled with differentially protected aminoglucose **14**, giving adduct **15** (yield based on recovered reactants >80%). Reduction followed by hydroxyethylation gave **16**, which was successfully transformed to mustard **17** with preservation of fidelity. Though somewhat less stable than **13** to hydrolysis, this candidate is now undergoing extensive testing against a battery of bulged



Scheme 1. Preparation of spirocyclic mustard cross-linking agents.



Scheme 2. Preparation of glycosylated spirocyclic mustards.



sequences, the results of which will be reported in due course.

In summary, the first examples of alkylating agents for bulged DNA targets have been demonstrated. Though of only moderate affinity, they will pave the way forward for more effective analogs. Using these reagents, the ability to manipulate a significant microenvironmental target of DNA and perhaps RNA may become reality, adding to the tools of molecular biology at our disposal.

Acknowledgements

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