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Synthesis of *endo*-(3-azabicyclo[3.1.0]hex-6-yl)-methanol and derivatives as new geometric/charge mimics of glycosyltransfer transition states

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Abstract—The syntheses of azabicyclo[3.1.0]hexane transition state (TS) analogs are described. Cyclopropanation of N-Boc-3-pyrroline with ethyl diazoacetate afforded a 1.6:1 *exo*/*endo* ratio of the resulting ester. Reduction of the *endo*-isomer with $LiAlH_4$ yielded the alcohol which was phosphorylated, or iodinated to provide access to aglycon containing TS analogs.

Glycosyltransfer can broadly be defined as the formation or hydrolysis of glycosidic bonds, respectively catalyzed by glycosyltransferases and glycosidases. In most cases, the transition states (TS's) for these reactions feature a glycon ring which is flattened having substantial oxocarbenium ion character.[1] The aglycon leaving groups (nucleotides for transferases, other saccharides for glycosidases, or nucleobases for *N*-glycosidases) depart in a trajectory that roughly place them above or below the plane of the glycon oxocarbenium ion at the TS (Fig. 1A).[2] One application of the results of mechanistic study is to design new inhibitors for the target enzymes, and in particular, transition state analog inhibitors have considerable potential for tight binding.[3] A successful approach in the design of oxocarbenium ion transition state inhibitors of both *N*- and *O*-glycosidases has been to use an aza-sugar analog of the glycon, in which a basic nitrogen is installed at a position isosteric with either the anomeric carbon or ring oxygen.[4] Mimics of the aglycon may be directly attached to these molecules to further add selectivity and binding energy. Two possible limitations of this approach are that the attached aglycon mimic is connected at a sp^3 hybridized C– or N–, placing it in a position more coincident with the oxocarbenium ion plane than is likely to be the case in the enzymatic transition states, and the distance between the glycon and aglycon is too short or long, depending on whether the glycon and aglycon are directly attached or have a spacer atom/group between them.

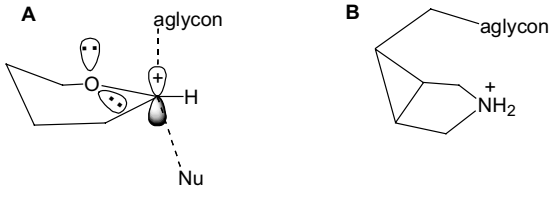

Figure 1. (A) A glycosyltransfer transition state. (B) Bicyclic charge and geometry transition state analogs.

We therefore sought to design transition state analogs (Fig. 1B) that would allow (i) mimicry of the position of an aglycon above or below the oxocarbenium ion plane; (ii) provide a transition state like distance between the aglycon and glycon mimics; and, (iii) allow for mimicry of an oxocarbenium ion via planarity, charge or both. In earlier work a [3.1.0]bicyclohexane-nucleotide (Fig. 2) was synthesized and found to be an effective inhibitor of sialyltransferase.[5] The synthetic route to the [3.1.0]bicyclohexane core utilized the known epoxidation and in situ rearrangement of norbornadiene.[6] We felt that this route would not lend itself for synthesis of aza analogs and thus sought an approach based on cyclopropanation of protected 3-pyrroline.[7] We discuss herein the efficient synthesis of a versatile group

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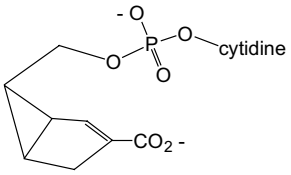


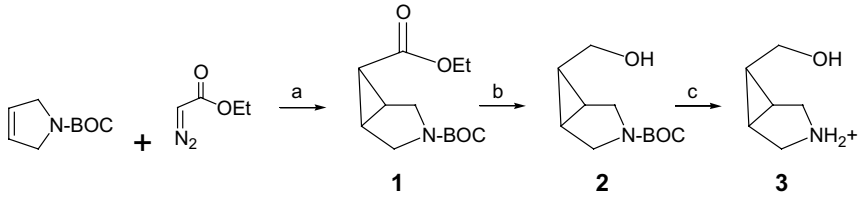
Figure 2. A geometric TS analog inhibitor of sialyltransferase. (Ref. 5).

of azabicyclo[3.1.0]hexane compounds that could mimic the structure and electrostatics of enzyme-bound transition states. In this letter we also report *endo*-6-hydroxymethylaza[3.1.0]bicyclohexane; in previous work only the *exo*-isomer has been reported.[7]

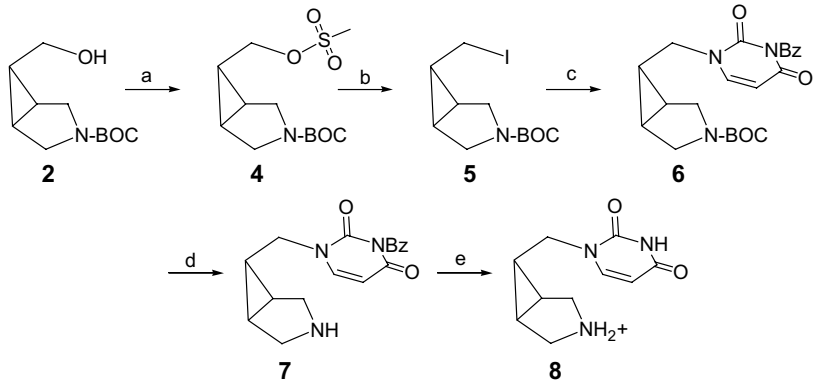
We started with Boc-protected 3-pyrroline for cyclopropanation with ethyl diazoacetate which differed slightly from the prior use of CBz-protection.[7] Both rhodium acetate and Evan's bisoxazoline ligand and copper triflate were explored as potential catalysts,[7–10] with the former affording the better yield. After slow addition of ethyl diazoacetate via syringe pump, the catalyst was removed by passing the mixture through a bed of Celite. A two-column chromatographic purification (silica, 10:1 toluene/EtOAc, then 20:1 toluene/EtOAc) afforded *endo*-**1** in 23% yield (the *exo*:*endo* ratio of isolated products was 1.6:1). Assignment of the identity of the *endo*-isomer was based on comparison with literature data.[7] The proton NMR chemical shifts of the *endo* and *exo* ester compounds **1** differ significantly for the ring fusion protons. The ring fusion protons for *exo* appear at 2.05 ppm as a broad multiplet, whereas those for the *endo*-isomer appear at 1.82 ppm. While the bridgehead cyclopropyl hydrogen for the *exo*-isomer is buried under the Boc-group hydrogens, the *endo*-isomer's bridgehead hydrogen appeared at 1.70 ppm as an apparent dd. Compound *endo*-**1** was reduced smoothly with $LiAlH_4$ in refluxing THF to afford alcohol **2** in 91% yield. The removal of the Boc group in TFA/CH_2Cl_2 provided a quantitative yield of compound **3** (Scheme 1).

The Boc-protected intermediate **2** was mesylated to afford **4** in 83% yield after workup and chromatography on silica. Mesylate **4** was iodinated in dry acetone for 2 h. However iodide-**5** was not isolated due to its lability. Addition of *N*-3-benzoyluracil to **5** in DMSO/DMF (1:8) using K_2CO_3 as base catalyst[11] afforded **6** in 82% yield after filtration of the reaction mixture through Celite and purification by silica column chromatography. After TFA deprotection to remove the Boc group, **7** was refluxed in 6 N HCl for debenzoylation.[12] The aqueous reaction mixture was washed with benzene then ether to remove all benzoic acid byproduct from the deprotection, then neutralized and extracted into chloroform to afford compound **8** in 97% yield, which was homogeneous by ^1H NMR and TLC (Scheme 2).

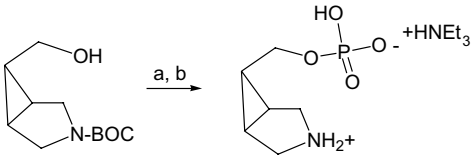
Compound **2** was *O*-phosphorylated in neat $P_2O_3Cl_2$, then purified on Dowex-1 (HCO_3^-) (0–0.5 M $NaHCO_3$ gradient) following a procedure developed in this laboratory.[13] Eluted product-containing fractions were detected with the malachite green assay.[14] The fractions were combined and desalted with Amberlite IR-120 (H^+) resin to provide **9** in 22% yield. The ^{31}P NMR spectrum showed a single resonance that appeared as a complex triplet at δ 1.1 ppm in gated decoupled spectra[15] (Scheme 3).



Scheme 1. Reagents and conditions: (a) $Rh_2(OAc)_4$, CH_2Cl_2, rt, 48 h, 38% *exo*, 23% *endo*; (b) $LiAlH_4$, THF, reflux, 12 h, (91%); (c) TFA, CH_2Cl_2, 0° to rt, 3 h, quantitative.



Scheme 2. Reagents and conditions: (a) MsCl, Et_3N, CH_2Cl_2, 0° to rt, 12 h, 83%; (b) NaI, acetone, rt, 2 h, quantitative; (c) N3-benzoyluracil, K_2CO_3, 1:8 DMSO:THF, rt, 42 h, 82%; (d) TFA, CH_2Cl_2, 3 h, 97%; (e) 6 N HCl, reflux, 42 h, quantitative.



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Scheme 3. Reagents and conditions: (a) $P_2O_3Cl_4$, 0°, 5h; (b) Et_3NH^+-HCO_3^-, H_2O pH7.

In a preliminary study, compound **8** was found to inhibit *Escherichia coli* uracil DNA glycosylase (UDG) by 30% at 1 mM in an assay against 1 mM slow substrate[13] 3′,5′-deoxyuridine diphosphate. Because UDG greatly prefers binding oligonucleotide substrates and requires recognition of flanking phosphate groups,[16,17] the inhibition observed with this very simple analog is noteworthy. Compounds related to **8** may be useful for inhibition of other *N*-glycohydrolases which proceeds via oxocarbenium transition states. Further application includes incorporation of the aza-bicyclohexane core into RNA and DNA for application against *N*-glycosylases that act upon nucleic acid substrates.

In summary, we have designed and synthesized a new scaffold for glycosyltransfer transition state analogs which mimic the electrostatics and the trigonal pyramidal geometry of the transition state. Present work involves elaboration of the phosphorylated compound **9** to afford glycosyltransferase inhibitors. It is also conceivable that with a suitable functionality attached to the exocyclic bridgehead methylene group of compound **3**, we will be able to either provide interaction with the general base that assists the glycon acceptor's attack from the backside of the sugar plane, or mimic the incoming nucleophilic group of the acceptor itself. Thus, such molecules may be used in tandem with the presence of the different leaving group moieties to serve as a potent synergistic inhibitor.

Acknowledgements

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15. Selected data for **1**: ^{1}H NMR *exo*-isomer: ($CDCl_3$) δ 4.10 (q, 2H), 3.68 (d, 1H), 3.60 (d, 1H), 3.40 (m, 2H), 2.05 (m, 2H), 1.45 (t, 1H), 1.40 (s, 9H) 1.25 (t, 3H). *endo*-Isomer: ($CDCl_3$) δ 4.10 (q, 2H), 3.77 (dd, 2H), 3.48 (br t, 2H), 1.83 (m, 2H), 1.72 (dd, 1H), 1.40 (s, 9H), 1.22 (t, 3H). HRMS (EI pos); Calculated for $C_{13}H_{21}N_1O_4$ (M+) 255.1470; found: 255.1466. For **8**: ^{1}H NMR (D_2O) δ 7.66 (d, 1H), 5.84 (d, 1H), 3.75 (d, 2H), 3.45 (br s, 4H), 2.0 (br s, 2H), 1.05 (m, 1H). HRMS (EI pos); Calculated for $C_{10}H_{14}N_3O_2$ (M+) 208.1086; found: 208.1089. For **9**: ^{1}H NMR (D_2O) d 3.81 (t, 2H), 3.47 (s, 4H), 1.91 (br s, 2H), 1.22 (m, 1H). ^{31}P NMR (D_2O) 1.10 (apparent t).
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