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Synthesis of 9-(4-Thioxylofuranosyl)adenine via a Novel Glycosylation Reaction

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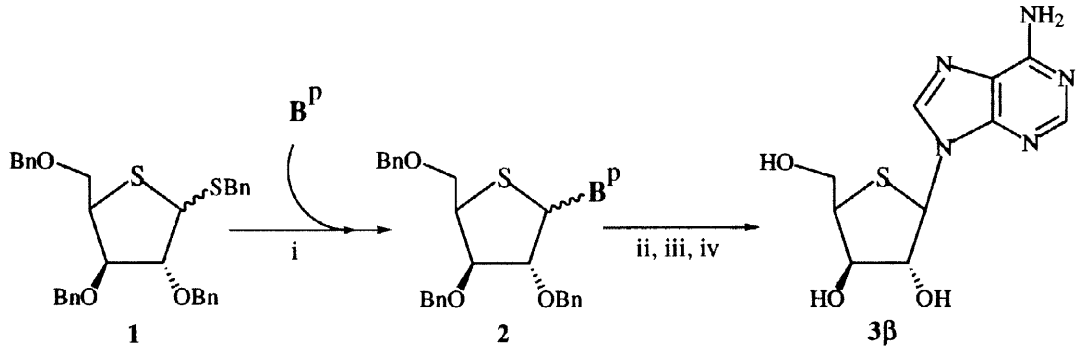
Abstract. 9-β-(4-Thioxylofuranosyl)adenine was synthesized through direct glycosylation of adenine with a 4-thio-2',3',5'-tri-O-benzyl-1-thiobenzyl intermediate. We report a novel, streamlined synthetic pathway to the targeted compound, involving direct addition of a poor nucleophile to a thioether intermediate. Based on our initial observations, we propose an iodium ion promoted elimination/addition reaction mechanism.

4-Thionucleosides have been studied extensively over the past ten years because of their anti-viral activity and potential utility as components of anti-sense oligonucleotides.[1,2] A study of nucleoside analogs possessing anti-inflammatory activity generated our interest in 4-thionucleosides, particularly 9-β-(4-thioxylofuranosyl)adenine (3β).[3] To the best of our knowledge this report details the first synthetic preparation for the 4-thioxylofuranosyl isomer of adenosine.

In several attempts to produce the targeted compound using procedures developed for the construction of 4-thioribonucleosides, we encountered deficiencies in both the overall yield and stereoselectivity of the synthesis. Imbach and coworkers reported a multistep synthesis for 4-thioadenosine, but the chemistry resulted in low yields of the purine β isomer.[4] Improved stereoselection was achieved by Montgomery and coworkers at the expense of additional synthetic steps and glycosylation methods using purine analogs other than adenine.[5] We therefore sought to develop a more direct route to the desired analog that would proceed through direct displacement of a thioether by adenine (Scheme 1). By employing conditions similar to those used for disaccharide formation[6], we have successfully produced 4-thioxyloadenosine (**3**) in a 34:20 (β:α) ratio with better overall yield and shorter synthetic preparation than methods previously reported for the synthesis of the 4-thioribonucleoside derivative.



1 2 3β

Scheme 1. B^P = N6-benzoyl adenine, i = NIS,TfOH, CH_2Cl_2: $(Et)_2O$ (1:1), ii = BBr_3, CH_2Cl_2, iii = 8M ammonia in methanol, iv = RP-HPLC.

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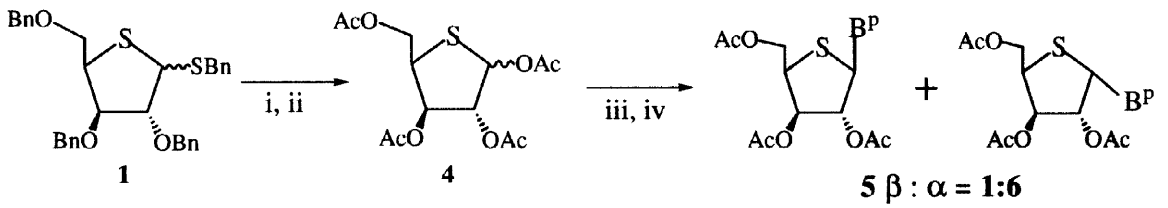
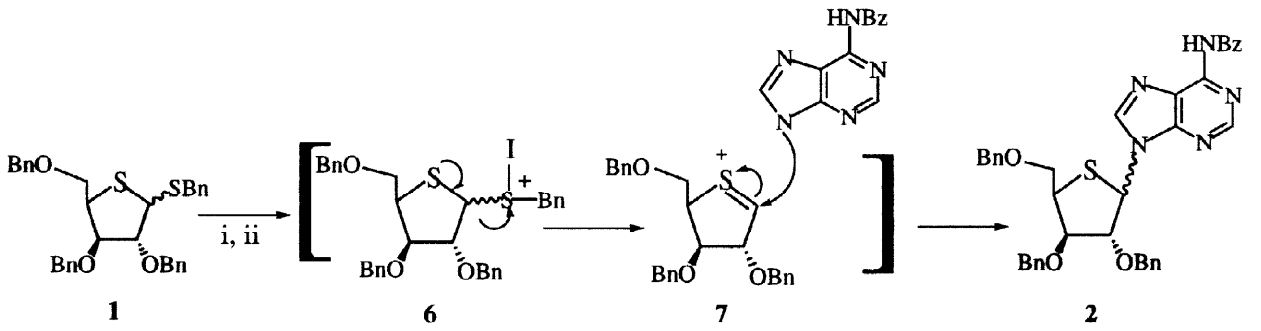
Iodium ion promoted disaccharide formation in the presence of triflic acid has been well studied with a number of different glycosides[6]. N-iodosuccinimide in the presence of an organic acid efficiently promotes the glycosidation of acylated thioglycosides with a glycosyl donor to give the stereoselective 1,2 trans linked disaccharide. We have found that this system works efficiently for the glycosylation of adenine with 4-thioxylofuranose. However, unlike the disaccharide forming reaction we found that the formation of the thionucleoside derivative did not proceed with stereoselectivity.

In our application of previously reported procedures for 4-thioribonucleosides to this analog, the benzylated sugar (**1**) was deprotected with boron tribromide and the 1-thiobenzyl group was removed with mercuric acetate/acetic acid to produce the reprotected tetra-O-acetyl derivative (**4**) as outlined in Scheme 2. We observed a 6:1 β:α ratio, consistent with previous literature reports[7] for the 4-thioribofuranosyl derivative. Subsequently, when standard glycosylation conditions were applied, the purine attacked through an S_N2 type mechanism, resulting in predominately the inversion product α isomer.



Scheme 2. i = BBr_3 in DCM at -70 °C, ii = $Hg(OAc)_2$ and AcOH, iii = BSA, iv = N6-Bz adenine and TMSTF.

We hoped to create a more efficient synthetic route by eliminating the transient protection step and forming the 4-thioxyloadenosine directly from the fully protected 1-thiobenzyl-4-thioxylosugar (**1**) via displacement of the thiobenzyl moiety. Also, if addition of adenine proceeded through a S_N2 type displacement, with inversion of configuration, one would expect to yield a significant improvement in β isomer yield. Compound **1** was prepared essentially as reported by Leydier *et al* except that D-xylose was used as the starting material.[4] Several modifications were introduced to significantly improve the overall yield.[8] A wide variety of conditions for direct addition with concomitant displacement of the thiobenzyl group were attempted with no success. In addition, oxidation of the thiobenzyl mercaptan to the sulfoxide followed by displacement was attempted, but did not result in formation of the desired product. When (**1**) was treated with N-iodosuccinimide and catalytic triflic acid followed by addition of N6-benzoyl adenine, a 34:20 (β:α) ratio of nucleoside (**3**) was observed after deprotection. One would predict that addition of adenine to (**1**) would result in predominately **2**β if the reaction proceeded through a S_N2 type mechanism because the preparation of precursor (**1**) yields predominately the α isomer according to NMR analysis.[9] Based on the ratio of isomers obtained under our reaction conditions, we propose an elimination/addition mechanism of action analogous to the mechanism of thioketal deprotection as outlined in Scheme 3.



Scheme 3. i = N6-Bz adenine, ii = NIS, TfOH.

It is likely that the iodium ion attacks the thiobenzyl sulfur, forming a transient ionized sulfur intermediate (**6**). Unlike the previously reported disaccharide forming reaction in which the activated intermediate is directly displaced by the incoming monosaccharide, we propose that the ring sulfur then contributes lone pair electrons to eliminate the 1-thiobenzyl group. The transient thioketal intermediate (**7**) thus generated is attacked by the purine base at the 1-position, resulting in the fully protected 4-thioxylonucleoside (**2**) anomers. Further, more detailed mechanistic studies such as solvent polarity influence on product ratios will be necessary to fully elucidate the mechanism of the reaction.

The glycosylation was carried out by adding 1.2 equivalents of N-iodosuccinimide and 0.5 equivalents of triflic acid to a mixture of protected thiosugar **1** (0.04 mmol, 21 mg) and N6-benzoyl adenine (0.05 mmol, 6.8 mg) in dichloroethane:ether (1:1) at 0°C. The reaction was allowed to proceed for 10 hours and resulted in the formation of 0.035 mmol of **2** (10 mg, 87.5 %). The benzyl protecting groups were removed with boron tribromide in dichloromethane at -70°C followed by base exocyclic amine deprotection in 8M ammonia in methanol. We found it essential to remove the benzyl groups from the sugar hydroxyls before deprotecting the base. When the N6-benzoyl group was removed from the base first, we observed acid catalyzed depurination during the removal of the benzyl groups from the sugar. The fully deprotected **3α** and **3β** isomers of 9-(4-thioxylofuranosyl)adenine were separated by HPLC[10] and characterized by mass spectrometry, ^{1}H NMR and 2-D NMR spectroscopy.[11] The overall yield of **3β** after purification was 0.022 mmol (6.3 mg).

In summary, we have devised a synthetic pathway to 9-β-(4-thioxylofuranosyl)adenine that may find general utility in the assembly of related adenosine analogs. The pathway has several potential advantages over currently employed routes to other 4-thio modified adenosine analogs such as the ribose and 2'-deoxyribose derivatives. These include fewer steps, higher overall synthetic yield and greater diastereoselectivity than those previously reported for the ribose derivative. Previously reported methods for construction of 4-thioadenosine involve a 10-step synthesis resulting in a 21% overall yield and 5:18 (β:α) ratio.[4] We have devised an 8-step synthesis to the novel 9-(4-thioxylofuranosyl)adenine (**3**) resulting in a 30% overall yield and a 34:20 (β:α) ratio. Application of this synthetic scheme to the construction of the 4-thioribofuranosyl and 2'-deoxy-4-thioribofuranosyl adenine analogs could be of significant benefit in deriving these important components of oligonucleotide analogs. In addition, this route may find use for the addition of a variety of poor nucleophiles to the anomeric position of thiosugars.

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8. An inversion of configuration at the 4 position was necessary to attain the D- xylo isomer after ring closure. This was achieved through a Mitsunobu inversion reaction. We found the reaction proceeds more efficiently when the solvent employed was benzene, rather than the commonly reported THF. The diethylazodicarboxylate (DEAD) was replaced by the more reactive diisopropylazodicarboxylate (DIAD). The DIAD (1.5 eq) was added dropwise over five minutes to a mixture of 2,3,5-tri-O-benzyl-xylose-1-dithiobenzylacetal (0.357 mmol, 0.181 g), and 1.5 equivalents of both p-nitrobenzoic acid and triphenylphosphene under nitrogen in a vessel protected from light. Formation of the product was monitored by tlc and the product was purified by silica gel chromatography using hexane:ethyl acetate (5:1).
9. Intermediate (1) was HPLC purified and analyzed by mass spectroscopy and ^{1}H NMR. MS m/e 543 (M+H)+; 1HNMR (CD_3OD) 3.4 (m,1H,H-4), d 3.51 (dd, 1H, H-5), 3.75 (dd, 1H, H-5'), 4.2(m, 2H, H-2 and H-3), 4.35-4.45 (2S, 2H,-S-CH_2-C_6H_5), 4.5 (d, 1H,H-1), 4.6-4.72 (m, 6H, -O-CH_2-C_6H_5), 7.2(M, 20H, -O-CH_2-C_6H_5, S-CH_2-C_6H_5).
10. The 4-thioxylonucleoside was purified by reverse phase chromatography using C-18 silica as the solid phase (Waters DeltaPak) and water/methanol as the mobile phase. The sample was dissolved in H_2O:CH_3OH (1:2) before injection. A gradient of 0 - 70% methanol over 30 minutes was found to be effective in separating the isomers. The β isomer eluted at 18.8 minutes and the α isomer at 20.02 minutes.
11. Each isomer was analyzed by positive ion electrospray ionization (ESI) mass spectometry, ^{1}H NMR and 2D COSI NMR **3β** : MS *m/z* 284 $[M+H]^+$; ^{1}H NMR (CD_3OD) δ 3.68 (m,1H, H-5',5"), 3.89 (m, 1H, H-5",4'), 4.15 (dd, 1H, H-3'),4.59 (dd,1H, H-2'), 5.01 (t,1H, OH5'), 5.38 (d,1H, OH3'), 5.58 (d,1H, OH2'), 5.91(d, 1H, H1'), 7.19 (s, 2H, NH2), 8.75 (s, 1H, H2). **3α** : MS *m/z* 284 [M+H]+ ; ^{1}H NMR (CD_3OD) δ 6.16 (d, H, H1'). 2D data not shown.