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Radical cyclisation mediates the synthesis of a new base-ribose carbon bridged adenosine

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Abstract—Reaction of tributylstannyl, radical generated in situ with AIBN, with (2′,5′-di-O-*tert*-butyldimethylsilyl-3′-C-ethynyl-β-D-*ribo*-furanosyl)adenine (**5**) gave in 69% yield the new conformationally locked nucleosides **6** (*E*/*Z*).

Adenosine modified at the 3′β-position by an ethynyl group **1** was the first 3′β C-substituted adenosine substrate of adenosine deaminase (ADA, EC 3.5.4.4).[1] The fact that this compound is lesser substrate than adenosine might be explained by the pseudo-axial ethynyl group destabilizing the conformation requested to form an active substrate–enzyme complex (as shown in Fig. 1, compound **1**). Based on the structural data, it has been proposed that adenosine in the active complex adopts a conformation where the C-8 hydrogen of the base points in the direction of the pseudo-axially disposed C-3′β ribosyl hydrogen.[2] (North conformation[3] as in Fig. 1, compound **1**.) Conformationally locked nucleosides could confirm this proposal.

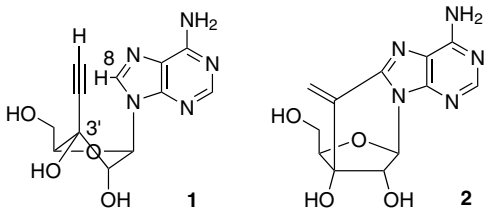

Figure 1. Representations of 3′-β-ethynyladenosine **1** in North conformation and of carbon bridged compound **2**.

In the recent years, conformationally locked nucleosides have attracted attention as therapeutic and diagnostic tools.[4–12] Major interest has focused on the chemical modification of the sugar moiety. For instance, adenosine analogues with carbocyclic rings locked in different conformations have been employed in order to identify the preferred sugar ring conformation for binding and catalysis with adenosine deaminase.[9,12]

In order to confirm this proposition, we undertook the synthesis of bridged adenosine derivative **2** where the base and the ribose are locked in a conformation that should be more favourable to form an active enzyme–substrate complex. Herein, we describe the first 3′,8 carbon bridged adenosine **2** which possesses the same functionalities[13] as adenosine and its biological evaluation on ADA. The synthesis of compound **2** is described in Scheme 1. The key step is the radical cyclisation similar to the one used on 3′β-ethynyl uridine[14] and is based on the known susceptibility of the N7–C8 bond to attack by carbon centered radicals.[13,15–23] The intermediates **4** (R_1, R_2 = H,) and **5** (R_1 = TBS, R_2 = H) used for our study were prepared from the known compound **3**[24] by established methods. Under free radical generating conditions four compounds were obtained: the **6** (*E*) and (*Z*) cyclo-adducts and the (*E*) and (*Z*) vinylstannyl-adducts **7** beside minor products not investigated here.

Keywords: Nucleoside; Adenosine; Deaminase; Locked; Conformation; Radical; AIBN; Tributyltin hydride; Purinergic receptors.
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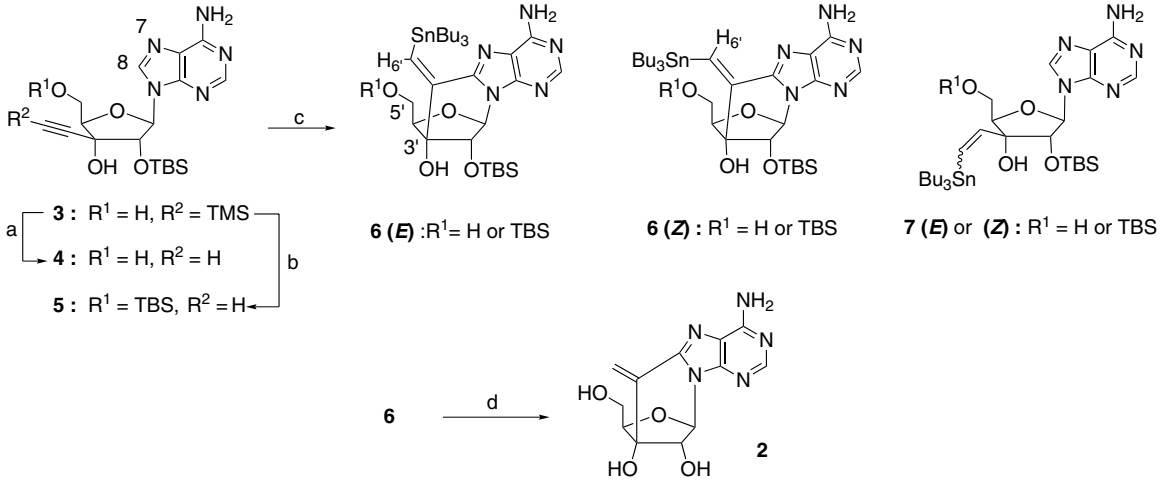

Scheme 1. Reagents and conditions: (a) K_2CO_3, MeOH, 93%; (b) (1) TBSCl, Py; (2) K_2CO_3, MeOH, 76% in two steps; (c) Bu_3SnH (2×2 equiv), AIBN (2×1 equiv), Tol, 80 °C, 24 h, 69%; (d) (1) Bu_4NF, THF; (2) HCl/MeOH (4%), 75% in two steps.

In order to optimise the yield of cyclic compounds, we varied three parameters. The group at C-5′ was either the free hydroxyl or the bulky *tert*-butyldimethylsilyl ether. The temperature and reagent concentration were varied.

With the hydroxyl at C-5′, the reaction gave the addition products **7** as the major compounds ($R^1 = H$; 74%: (*E*)/(*Z*) = 1/57). By contrast, with the silyl ether at C-5′, the cyclo-adducts **6** were favored ($R^1 = TBS$). Thus, we concentrated our efforts by using the protected intermediate **5** and chose to carry out the following experiments with AIBN as the initiator instead of Et_3B because of the easier control of the radical concentration.[25] The best results were obtained at 80 °C when an excess of tin hydride and AIBN[26] were used and the reaction lasted longer (24 h). Under these conditions[27] the conversion was completed, the ratio of products **6**/**7** and of (*E*)/(*Z*) isomers for **6** were the highest, 93/7 and 84/16, respectively. Whereas compound **6** (*E*) was easily purified by silica gel chromatography, the corresponding isomer **6** (*Z*) was difficult to isolate pure. Product **6** was isolated in 69% combined yield as an isomeric mixture. The ratio of (*E*)/(Z) products **6** was the largest when the reaction lasted the longest and the concentration of the starting compound **5** was the lowest, suggesting that (*Z*) to (*E*) isomerisation occurred.[28,29] However treatment of the derivative **6** (*Z*) in presence of AIBN/Bu_3SnH in toluene at 80 °C did not show any detectable (*E*) isomer.

The **6** (*E*) and (*Z*) cyclo-adducts were characterized by UV, $[\alpha]_D$, ^1H and ^{13}C NMR and HRMS.[30] The configuration of the double bond of the cyclic isomers **6** was determined by ROESY experiments. When the olefinic proton H-7′ was saturated, marked Overhauser effect with the proton of the C-3′ hydroxyl and significant NOEs with protons 5′ and 2′ were observed for the (*E*) isomer; whereas, no NOE was observed for the (*Z*) isomer.

Destannylation of compounds **6** was attempted with BuLi in THF and acetic acid in different solvents but none of these methods was satisfactory.[31,32] Finally, after deprotection of the silyl moieties with TBAF in THF, tin removal was accomplished by treatment in a mixture of concd HCl in MeOH (4%).[32] The desired compound **2** was isolated by centrifugation as the chlorhydrate in 75% yield in two steps.[33]

The cyclic compound **2** was tested on ADA and has shown that it was neither a substrate nor an inhibitor of ADA. A steric clash between the side chains of Asp 19 and/or Phe 65 and the methylidene group in bridged adenosine **2** is a plausible explanation to the fact that the compound does not bind to ADA (see Ref. 2). In another hand, a more intriguing result was obtained with 3′-β-ethynyl-*ribo*-furanosyl-purine, which was prepared as a potential transition state inhibitor by structural analogy to other known inhibitors of ADA.[2] This compound, that lacks the six-amino group, was tested on ADA and has shown to be not an inhibitor whereas compound **1** was substrate of the enzyme. The latest observation brings up at least one more question. Does the model proposed for the active enzyme–substrate complex suffer exceptions or is it correct?

In conclusion, a new base-ribose locked adenosine derivative was synthesized by radical cyclisation. By further chemical manipulation, our synthetic approach should allow rapid access to an original class of compounds amenable for biological evaluations. Stille type palladium–catalyzed coupling reactions may be useful to enlarge the structural diversity. This new locked cyclo-adenosine resistant to ADA could represent a new and attractive scaffold to be tested on purinergic receptors for instance.[5,7,8]

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27. Typical procedure: Nucleoside **5** (0.20 g, 0.38 mmol), Bu_3SnH (2 equiv, 0.21 mL, 0.77 mmol) and AIBN (1 equiv, 64 mg, 0.38 mmol) were added successively in toluene (20 mL, *c* 0.02 M in nucleoside). The reaction mixture was then degassed three times and heated to 80 °C under Ar. After 4 h stirring, another 2 equiv of Bu_3SnH and 1 equiv of AIBN were added. After 24 h, the volatiles were eliminated under reduced pressure. The ratio of the different products was deduced from ^1H NMR of the crude extract. The oily crude extract was dissolved in $CHCl_3$ and purified by silica gel chromatography with ether/hexane (55:45 to 70:30). Whereas compound **6** (*E*) (R^1 = TBS) was easily purified by silica gel chromatography, the purification of the (*Z*) isomer **6** (R^1 = TBS) proved to be more tedious. Combined yield: 69%.
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30. (*E*)-1-[2′,5′-Di-*O*-*tert*-Butyldimethylsilyl-8,3′-(2-(tributylstannyl)ethylidene)-β-D-*ribo*-furanosyl]adenine **6** (*E*) UV (CH_3OH): λ_{max} = 304 nm, ε = 12100 $M^{-1}cm^{-1}$, $[\alpha]_D^{25}$ +35 (*c* 0.6, CH_3OH), ^1H NMR (200 MHz, $CDCl_3$) δ: 8.35 (1H, s), 6.92 (1H, s (84%), dd (16%), J_{SnH} = 54.5 and 52 Hz), 6.03 (1H, s), 5.39 (2H, s), 4.3 (1H, t, $J_{H4'H5'}$ = 6.5 Hz), 4.08 (1H, s), 3.52 (2H, m), 2.89 (1H, s), 1.41–1.58 (6H, m), 1.17–1.37 (6H, m), 0.85–1.16 (33H, m), 0.23 (3H, s), 0.22 (3H, s), 0.0 (3H, s), −0.04 (3H, s), ^{13}C NMR (50 MHz, $CDCl_3$) δ: 155.0 (q), 152.9 (t), 148.8 (q), 147.3 (q), 139.5 (q), 136.5 (q), 120.0 (t), 83.4 (t), 82.9 (t and q), 78.9 (t), 63.7 (s), 29.4 (s), 27.4 (s), 25.8 (p), 25.7 (p), 18.2 (q), 13.7 (p), 12.1 (s), −4.5, −5.3, −5.4 (p), HRMS: calculated: 806.3827, measured: 806.3833.
(*Z*)-1-[2′,5′-di-*O*-*tert*-Butyldimethylsilyl-8,3′-(2-(tributylstannyl)ethylidene)-β-D-*ribo*-furanosyl]adenine **6** (*Z*) UV (CH_3OH): λ_{max} = 310 nm, ε = 13600 $M^{-1}cm^{-1}$, $[\alpha]_D^{25}$ +38 (*c* = 0.7, CH_3OH), ^1H NMR (200 MHz, $CDCl_3$) δ 8.34 (1H, s), 7.57 (1H, s (84%), dd (16%), J_{SnH} = 47.5 and 46 Hz), 6.03 (1H, s), 6.06 (1H, s), 5.56 (2H, s), 4.23 (1H, dd, $J_{H4'H5'a}$ = 3 Hz, $J_{H4'H5'b}$ = 8.7 Hz), 4.05 (1H, s), 3,8 (1H, dd, $J_{H5'aH4'}$ = 3 Hz, $J_{H5'aH5'b}$ = 11, 7 Hz), 3.3 (1H, dd, $J_{H5'bH4'}$ = 8.7 Hz, $J_{H5'bH5'a}$ = 11.7 Hz), 2.88 (1H, s), 1.41–1.58 (6H, m), 1.17–1.37 (6H, m), 0.85–1.16 (33H, m), 0.25 (3H, s), 0.21 (3H, s), −0.06 (3H, s), −0.13 (3H, s), ^{13}C NMR (50 MHz, $CDCl_3$) δ: 155.8 (q), 153.8 (t), 149.7 (q), 147.3 (q), 140.1 (q), 137.1 (t), 105.2 (q), 86.9 (t), 83.6 and 82.8 (t and q), 80.1 (t), 64.9 (s), 30.6 (s), 30.1 (s), 28.4 (s), 26.8 (p), 26.7 (p), 19.3 (q), 19.1 (p), 16.3, 14.7 (p), 13.4 (s), −3.5, −3.9, −4.3, −4.6 (p), HRMS: calculated: 806.3827, measured: 806.3834.
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33. 1-[-8,3′-ethylidene)-β-D-*ribo*-furanosyl]adenine hydrochloride **2**. To a solution of the (*E*/*Z*) mixture of **6** (67 mg, 0.08 mmol) in THF (2 mL) was added Bu_4NF (105 mg, 0.33 mmol). After 1 h, the reaction was completed as evidenced by TLC ($CHCl_3$/EtOH: 9/1). The reaction mixture was filtered on a silica gel column ($CHCl_3$/EtOH: 9/1). After elimination of the volatiles, the product was treated with concd HCl (0.12 mL) in MeOH (3 mL) and the reaction was left under stirring overnight. The precipitate **2** was recovered by centrifugation, washed with MeOH (3×1 mL) and vacuum dried. Yield: 75%. ^1H NMR (D_2O/DMSO-d_6) δ: 8.22 (1H, s), 6.33 (1H, s), 6.02 (1H, s), 5.73 (1H, s), 4.27 (1H, m), 4.08 (1H, s), 3.47 (1H, m), 3.05 (1H, m), ^{13}C NMR (D_2O/DMSO-d_6) δ: 155.9 (q), 153.0 (q), 152.9 (t). 151.8 (q), 139.5 (t), 123,7 (q), 105.2 (s), 88.3 (t), 88.2 (q), 81.4 (t), 65.9 (s), (HRMS): calculated: 292.1045, measured: 292.1048.