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Tetrahedron 60 (2004) 6609–6617
Tetrahedron

Novel nicotinamide adenine dinucleotide analogues as selective inhibitors of NAD+-dependent enzymes

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Received 8 March 2004; revised 7 May 2004; accepted 27 May 2004

Available online 19 June 2004

Abstract—Three novel dinucleotide analogues of nicotinamide adenine dinucleotide (NAD^+) have been synthesised from D-ribonolactone. These compounds incorporate a thiophene moiety in place of nicotinamide and are hydrolytically stable. They have been evaluated as inhibitors of adenosine diphosphate ribosyl cyclase, glutamate dehydrogenase and Sir2 acyltransferase activities. Enzyme specificity and a high level of inhibition was observed for the dehydrogenase.
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1. Introduction

C-nucleoside analogues of ribosyl nicotinamide (Fig. 1) have been widely studied and some are currently in clinical trials.[1] They are endowed with several biological effects ranging from anti-tumour activities to inhibition of G-protein mediated cellular mechanisms.[2–4] Some of these effects have been directly related to the capacity of their adenine dinucleotide derivatives at inhibiting inosine monophosphate dehydrogenase.[2,5,6] Yet, C-nucleosides, such as ribosyl benzamide and tiazofurin (Fig. 1) can be inhibitors of other nicotinamide adenine dinucleotide (NAD^+) dependent enzymes, such as oxidoreductases, glycohydrolases and transferases, after their conversion to dinucleotide cofactor analogues. For instance, BAD, the NAD^+ analogue incorporating benzamide is a potent inhibitor of adenosine diphosphate (ADP) ribosyl cyclase, an important regulatory enzyme involved in the production of a modulator of Ca^{2+} concentration in cells.[7] Consequently, whilst most of these compounds act as potent anti-tumour agents by shutting down the guanosine monophosphate synthesis, they are also highly toxic to healthy cells.

Thiophenfurin, the thiophene homologue of ribosyl nicotinamide, was shown to possess good selectivity towards tumour cells in vitro and high-level of conversion to its dinucleotide form. Yet, in vivo, this derivative was found to be toxic.[8] Unspecific enzyme inhibition and metabolic modifications of thiophenfurin might be responsible for such an outcome.

Consequently, in order to optimise enzyme and cell selectivity and maintain high levels of inhibition, novel C-nucleosides that possess particular structural features for specific recognition are required. Many dinucleotide-binding enzymes bind NAD^+ via a fold consisting of two mononucleotide-binding motifs.[9] When one considers the

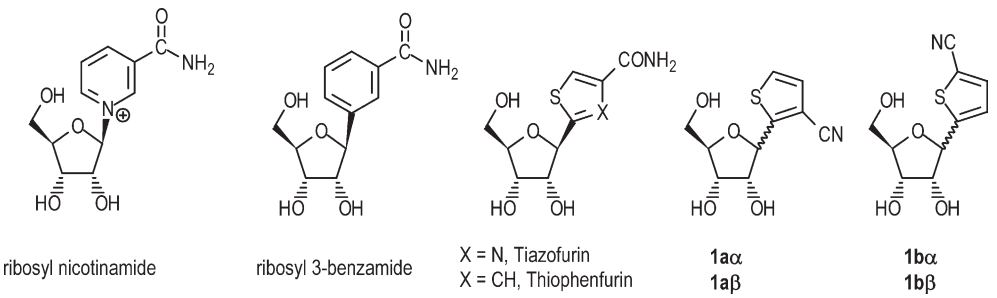

Figure 1. Ribosyl nicotinamide and analogues.

Keywords: C-nucleosides; Dinucleotides; NAD^+ analogues.
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0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.05.091

structural features of all the ribosyl nicotinamide analogues synthesised thus far, one notices that the amide moiety is a conserved functionality. Indeed, based on the available crystallographic data of enzymes co-crystallised with C-NAD^+ analogues, the amide moiety is involved in important hydrogen bonding interactions, acting as a hydrogen bond donor.[10–13] Consequently, a slight modification of this important structural feature might result in an abolition of binding when such interaction accounts for much of the recognition and stabilisation of the inhibitor–enzyme complexes. Meanwhile, such modification might provide the means to improve selectivity. We aim to establish whether selective enzymatic recognition can be observed amongst different classes of NAD^+-dependent enzymes by exchanging the amide moiety for an isoelectronic group capable of hydrogen bond interactions. As such, a nitrile group can be viewed as an isosteric group to an amide, capable of engaging in hydrogen bond interactions with an enzyme-binding pocket as a hydrogen-bond acceptor. To the best of our knowledge, there are no nitrile containing C-nucleosides, analogues of ribosyl nicotinamide thus far reported in the literature. Considering the good selectivity for tumour cells showed by thiophenfurin and the possibility of improving upon enzyme specificity by modifying the amide moiety, we have synthesised four novel C-nucleoside analogues (**1aα/β** and **1bα/β**) that incorporate a thiophene residue substituted by a nitrile group (Fig. 1). Three of these C-nucleosides have been converted to their dinucleotide parents and evaluated against three NAD^+-dependent enzymes that catalyse different types of reaction.

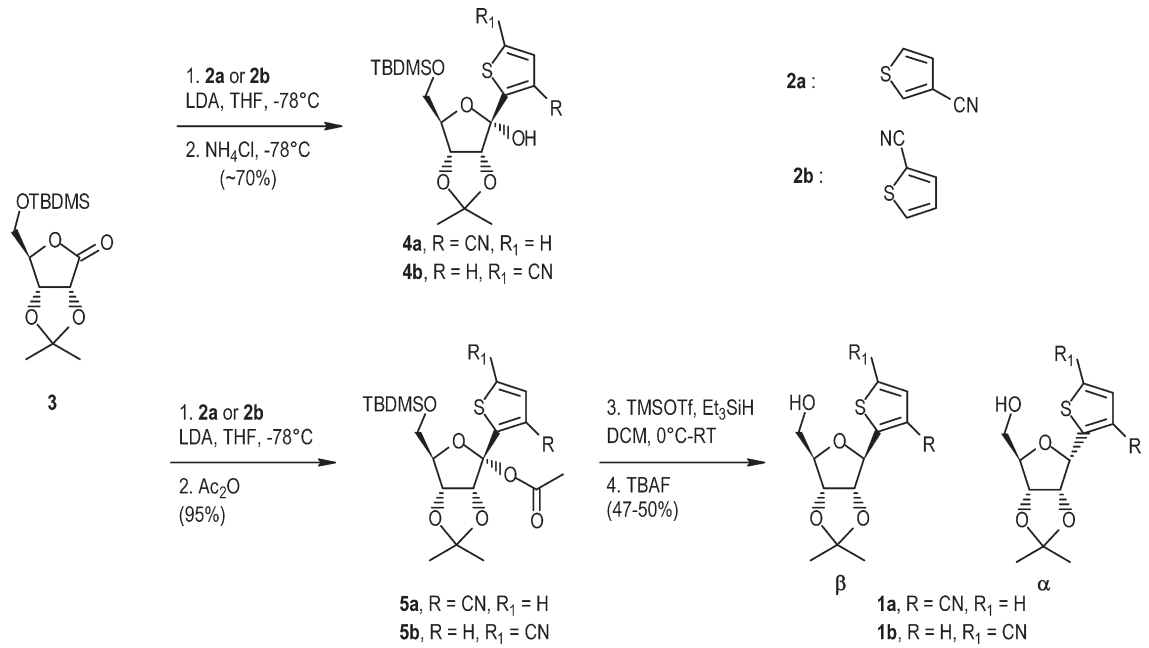
2. Results and discussion

2.1. Chemistry

Franchetti et al. have reported the synthesis of 2-(α/β)-D-ribofuranosylthiophene-3-carboxamide and 5-(α/β)-D-ribofuranosylthiophene-3-carboxamide using classical Friedel–Crafts' conditions starting with the tetraacetate ribofuranose and the appropriate ethyl thiophene carboxylates.[8] When applied to carbonitrile thiophenes, this method failed to yield any C-glycosylation product. Attempts to introduce any other types of thiophenes, including the ethyl thiophene carboxylate employed by Franchetti and thiophene itself, either led to no product formation or polymerised unidentifiable material.

Consequently, the addition of the lithiated derivatives of thiophenes **2a** and **2b** to the easily accessible 5-*O*-*tert*-butyldimethylsilyl-2,3-*O*,*O*-isopropylidene-D-ribono-1,4-lactone **3**,[14] followed by removal of the hydroxyl group in position 1′ of lactol **4** was employed.[15,16] (Scheme 1) The thiophene-3 (and -2)-carbonitrile **2a** (and **2b**), treated with LDA in THF, were reacted with lactone **3** for 30 min at −78 °C to form the lactol. Quenching of the reaction at −78 °C by addition of a solution of saturated NH_4Cl allowed the isolation of the hemiketals **4a** and **4b** as single isomers. Allowing the reaction to warm up or increasing the reaction time led to the opening of the sugar rings to form the ketones. The ketones were easily identified by ^1H NMR; for instance, δH-4′ shifted from 4.46 ppm of **4b** to 3.70 ppm in the ketone. A shift of the thiophene protons was also observed; δH-3 and δH-4 were 7.47 and 7.10 ppm for **4b**, respectively and 7.94 and 7.57 ppm for the corresponding ketone. This observation is in agreement with the strong deshielding effect a carbonyl has on aromatic protons. While combinations of silane and Lewis acids are known to reduce hemiketals, attempts to carry out direct dehydroxylation of **4a** and **4b** were unsuccessful. The chemical instability of similar carbohydrates towards such reaction conditions had been previously observed.[16,17]

Townsend had reported a procedure involving an acetylated hemiketal intermediate prepared in situ by trapping of the



Scheme 1. Synthesis of carbonitrile thiophene C-nucleoside analogues.

alkoxide, addition product of the lithiated aryl onto the lactone, with acetic anhydride at −70 °C.[18] This two-step reaction yielded the acetylated hemiketals **5a** and **5b**, quantitatively. It should be noticed that unlike previously reported, only one single 'anomer' was formed. Attempts to identify the stereochemistry at C-1′ either by ^{1}H NMR and proton-proton nuclear Overhauser effect (NOE) difference experiments or by crystallographic analyses were unsuccessful. Therefore, the assignments of the anomeric configurations were based on the ^{1}H NMR-$\Delta\delta$ values of the isopropylidene methyl groups.[19] $\Delta\delta$ values superior to 0.20 ppm indicate that the acetate group is *cis* to the isopropylidene moiety.

The formation and isolation of a single isomer whether the arylation reaction is quenched with NH_4Cl or Ac_2O at low temperature is at odds with the results reported by Townsend[18] and by Dondoni.[16] Yet, Benhinda recognised that the condensation step was very sensitive to steric factors imposed by the incoming nucleophile,[17] which could explain the results described by Townsend. However, the nucleophile employed in the study by Dondoni, a thiazolyl anion, is isosteric to the thiophene-3 (and -2) -carbonitrile **2a** (and **2b**) used in the present study. When the tribenzylated ribonolactone was used instead of **3**, no addition product could be detected. Consequently, activation of the lactone and α-configuration of the product could be attributed to a complexation of the lactone oxygen atoms at position 1 and 2 by the lithium cation, thus puckering the ribonolactone ring such that only an axial nucleophilic attack can be favoured. In addition, it is expected that steric effects due to the isopropylidene substituent would direct the attack of the nucleophile to the less hindered face of the lactone. When 5-*O*-TBDPS-2,3-*O*,*O*-isopropylidene ribonolactone was used instead of **3**, the two anomers α and β were identified by ^{1}H NMR (2:1 ratio). Yet no ring-open form could be detected when the reaction was quenched with Ac_2O at low temperature, indicating that the two stereoisomers were addition but not rearrangement products. Such explanation should tally with Dondoni's results when thiazole was used as nucleophile, yet it did not, as Dondoni observed the formation of opened compounds. The electronic effect of the nucleophile must therefore be considered.

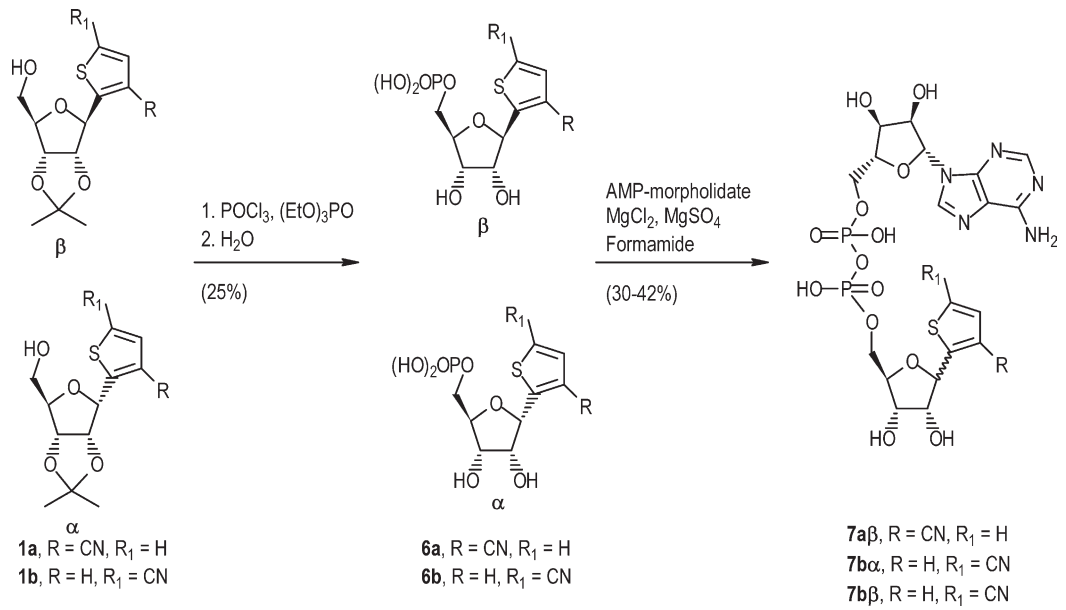
Since the thiophene-3 (and -2)-carbonitrile **2a** (and **2b**), both yield a single anomeric adduct product when reacted with **3**, it is highly probable that both reagents yield the kinetic addition products, which are also the most thermodynamically stable compounds under the reaction conditions. Compared with carbonitrile thiophenene, thiazole is a more electron-deficient aromatic. The formation of the thermodynamic product enol–ether intermediate in the α/β-rearrangement of the glycosylation product, obtained by addition of the lithiothiazole on **3**, might result from such a chemical characteristic. Such an enol is stabilised through conjugation between the thiazolyl substituent and the enol double bond.[16] Carbonitrile thiophenyl substituents, on the other hand, are sufficiently stable to not require extra conjugation that entails ring opening and electron delocalisation between the ketone and the thiophene ring. Indeed, no enol acetate was ever detected during this investigation. Consequently, in order to correlate the present results observed during the nucleophilic addition on lactone **3** to the literature precedents, one must consider the combination of effects due to the steric bulk of the nucleophile, the steric bulk of the 5′-substituent on the lactone, the activation of the lactone through complexation and finally the electronic nature of the nucleophile.

The subsequent removal of the acetoxy residue under Lewis acid conditions was optimised when TMSOTf and Et_3SiH in DCM were used in excess and the reaction was carried out over 30 min. Partial desilylation was detected and, consequently, after addition of triethylamine, TBAF was added to yield the desilylated C-nucleosides **1aα/β** and **1bα/β**. The C-nucleosides **1** were recovered in moderate yields (47–50%) after purification as the α and β isomers. The α/β ratio was always found to be in favour of the α isomer at 1.4/1 when TBDMS was used as protecting group at C-5′. The yields for this reaction were also slightly low when compared with those reported in the literature for similar C-glycosides.[15,17] The α and β anomers were identified by ^{1}H NMR NOE experiments as the anomeric protons were distinctively deshielded (δ=5.65 ppm for **1aα** versus δ=5.20 ppm for **1aβ**). When selectively irradiated, the signal of the α anomer **1aα** H-1′ gives an intensity enhancement of the H-2′ and H-3′ signals. The glycosylation position was determined by ^{1}H NMR. The NMR pattern for the aromatic protons of **1a**, a doublet, confirms the position of glycosylation of the thiophene at C-2. When the H-1′ signal of **1b** was irradiated, an NOE effect was observed at H-3.

Various conditions have been tried in order to increase yields and selectivity. Alternative Lewis acids, solvent and reducing reagents were considered, yet TMSOTf/Et_3SiH remained the only suitable combination. Competitive desilylation and removal of the isopropylidene group were thought to take place when $BF_3{\cdot}Et_2O$ was used as Lewis acid. Unidentifiable material was formed when DIBAH was used in combination with $AlCl_3$, while no reaction occurred with $Y(OTf)_3$. Finally, $SnCl_4$ yielded only the α-anomer products in poor yields. Optimised conditions were found to be 5 equiv. of Et_3SiH, 2.5 equiv. of TMSOTf in DCM starting the reaction at 0 °C and warming up to room temperature (Scheme 2).

The phosphorylation reaction of the C-nucleosides **1aα/β** and **1bα/β** was carried out following the Yoshikawa procedure,[7] this employing triethyl phosphate as solvent and activator. Addition of $POCl_3$ was done at room temperature and the reaction was monitored by HPLC (SAX column, $K_2HPO_4{\cdot}$50 mM, 5% MeOH, pH 3.5). Once the reaction complete, the excess of $POCl_3$ is quenched by addition of water. This resulted in the simultaneous removal of the isopropylidene group. Purifications of the C-nucleotides were achieved on AG-MP1 anion exchange resin using a gradient of TFA with an average recovery of 25%. The purification was not optimised.

The diphosphate linkage formation between **6aβ** and **6bα/β** and adenosine monophosphate employed the commercially available adenosine 5′-monophosphomorpholidate (4-morpholine-*N*,*N*′-dicyclohexylcarboxamidine salt).[20] The reaction was carried out in 4 days in a 0.2 M solution of $MnCl_2$ in formamide in presence $MgSO_4$ and was



Scheme 2. Dinucleotide analogues synthesis.

monitored by HPLC (SAX column, K_2HPO_4 50 mM, 5% MeOH, pH 3.5). Purifications of the three dinucleotides **7aβ** and **7bα** and **7bβ** were carried out on DEAE sepharose columns with a triethylammonium formate gradient. The isolated yields ranged from 30 to 42% after purification as calculated by phosphorus titration using Ames' assay.[21]

2.2. Enzyme inhibition

Franchetti has carried out extensive crystallography and computational studies on furafurin and thiophenfurin.[8] In his study, he observed that the S-atom remained *cis* to the furanose-oxygen and ab initio calculations suggested that this conformation was stabilised by an electrostatic interaction between a positively charged thiophene sulfur and a negatively charged furanose oxygen. Therefore, based on these observations, we could assume similar restricted rotation around the C-glycosidic bond of **7aβ** and **7bβ**. With such assumption, none of the C-dinucleotides are expected to bind like NAD^+ since the carbonitrile substituent will not be in an appropriate position to share hydrogen-bonding interactions with the amino acid residues that are involved in bonding interactions with the amide moiety of nicotinamide in NAD^+. Yet, the following results describe the effect of each of these C-NAD^+ analogues on three enzymes, each of which catalyses a distinct chemical reaction and each known to bind NAD^+ via a slightly different pattern.

2.2.1. Adenosine diphosphate ribosyl cyclase. Based on crystallographic data, this enzyme was thought to bind NAD^+ via the two nucleoside moieties (i.e., adenosine and ribosyl nicotinamide) with little binding stabilisation involving the pyrophosphate linkage.[22,23] Indeed this assumption was further supported by the fact that the cyclase catalyses the cyclisation of an NAD^+ derivative for which the pyrophosphate group was partially protected with *o*-nitro-phenol moieties.[24] Furthermore, nicotinamide mononucleotide (NMN) is a known potent inhibitor of the cyclase, while adenosine monophosphate is not.[12] This observation indicated that the initial recognition and binding of NAD^+ must occur at the northern ribose (NMN-end of NAD^+). Co-crystallisation of the cyclase with nicotinamide indicated strong hydrogen-bonding interaction between the amide moiety of nicotinamide and polar residues present in the binding pocket. As could be anticipated, when the three carbonitrile thiophene adenine dinucleotides **7aβ**, **7bα** and **7bβ** were evaluated against *Aplysia* ADP ribosyl cyclase in the presence of NAD^+ (data not shown), none of them was found to be an inhibitor. When high concentrations of C-dinucleotides were used (c.a. 500 μM, K_{mNAD}~ 120 μM), activation of the cyclase was clearly detected (data not shown). While such activation has never been reported for the *Aplysia Californica* cyclase, the human homologue, CD38, has been shown to be modulated by nucleotides such as GTP and ATP.[25,26] It is possible that at high concentration of C-dinucleotides, *Aplysia* cyclase is itself allosterically modulated.

2.2.2. Glutamate dehydrogenase. In the case of glutamate dehydrogenase (GDH) from *C. symbiosum*, NAD^+ is the co-factor which allows the oxidative deamination of L-glutamate to obtain the corresponding 2-oxo-glutarate. Baker et al.[27] showed that 'the dinucleotide is bound in an extended conformation with the nicotinamide moiety deep in the cleft between the two domains' of GDH. In this case, the NH_2 group of the nicotinamide in the *syn* conformation is involved in a hydrogen bond with the oxygen of the asparagine in position 240, while the hydroxyl group of threonine 209 interacts with the CO of the same nicotinamide moiety. On the other side, the enzyme provides a pocket for the adenine ring and several amino acids are involved in the stabilization of the ribose and the phosphate groups. These characteristics appear to be conserved in all the hexameric GDH sequences.

An initial screening of compounds **7aβ**, **7bα** and **7bβ** was performed in presence of a standard concentration of NAD^+ (1 mM) and L-glutamate (40 mM)[28] and showed the strongest inhibition effect for **7bβ** as reported in Figure 2.

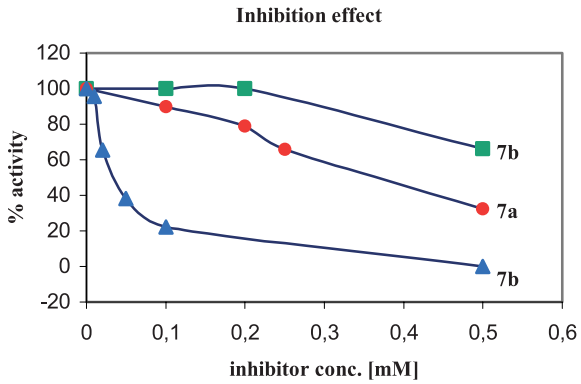


Figure 2. Effect of **7aβ**, **7bβ**, **7bα** on the specific activity (reported as 100% without inhibitor) of GDH.

Despite the fact that the concentration of 1 mM NAD^+ is ~10 fold the K_m, 0.5 mM of **7bβ** inhibited the activity of the enzyme almost completely. In marked contrast, **7bα** had very little effect.

A more detailed experiment was carried out with compound **7bβ** to define its K_i. In this case, the concentration of NAD^+ in solution was varied from 100 to 500 μM and for each concentration of NAD^+ a set of five concentrations of the inhibitor (0.1–5 μM) was tested. The reactions were performed at 25 °C in phosphate buffer 0.1 M at pH 7.0, and the activity was measured by following the formation of NADH at 340 nm (UV Spec, Cary 50). The results are shown in Figure 3.

From each experiment, a K_m value was calculated and plotted versus the concentration of the inhibitor in Figure 3 to obtain the inhibition constant K_i measured as the negative abscissa intercept (Fig. 4).

This elaboration yielded a K_i=1.09 μM for **7bβ**, confirming the strong inhibition effect produced by this NAD^+ analogue. From the observation of the V_{max} in Figure 2, we can also conclude that **7bβ** is a predominantly competitive inhibitor.

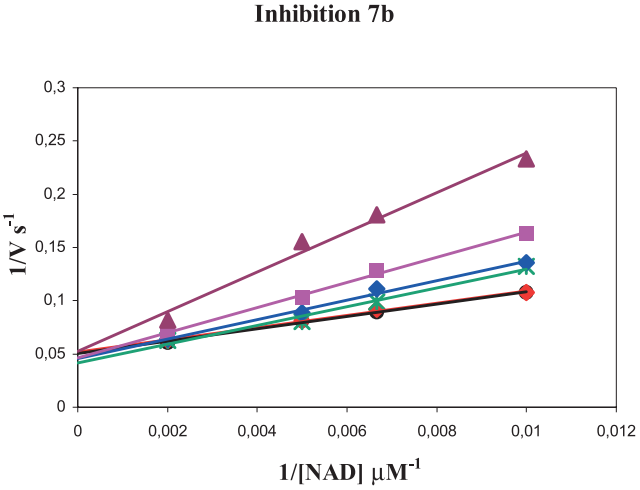


Figure 3. Lineweaver–Burk plot of the inhibition effect of **7bβ** on the GDH reaction. The inhibitor concentrations were 5 μM (▲), 1 μM (■), 0.5 μM (◆), 0.25 μM (✳), 0.1 μM (◆), and 0 μM (●).

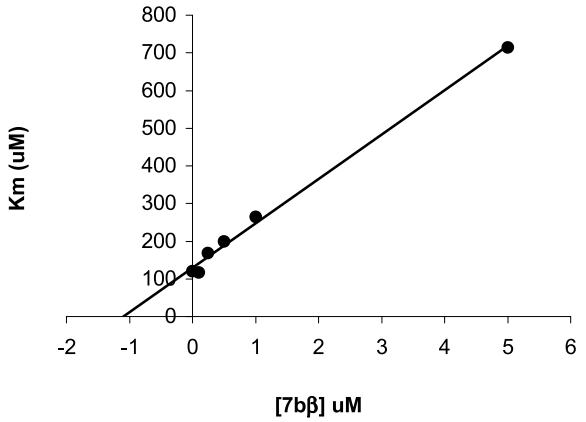


Figure 4. K_ms of **7bβ** obtained for different concentrations of the substrate.

2.2.3. Histone deacetylase, Sir2. Sir2 histone deacetylase has recently come under scrutiny when it was discovered that unlike most histone deactylase, it was an NAD^+ dependent enzyme.[29] The nature of the product of the reaction (1′-*O*-Ac- vs 2′-*O*-Ac-ADP) and the mechanism by which the products are formed are still controversial and under investigation. The assay methods currently available are also limited and while chromatographic analyses developed for the kinetic evaluations of *Aplysia* cyclase were initially deemed appropriate, the results obtained were inconsistent and no conclusive results could be drawn with regards to the effect of these dinucleotides on this deacetylase. We are currently optimising methods to assay for deacetylase activity more reliably.

3. Conclusion

Both β-NAD^+ analogues were selective inhibitors of the dehydrogenase, yet potency was dependent of the substitution pattern on the thiophene ring. Potent inhibition was obtained when the thiophene carbonitrile group could act as a hydrogen bond acceptor. To position the nitrile group and achieve appropriate binding, the electrostatic interactions between the positively charged thiophene sulfur and the negatively charged furanose oxygen must have been compensated for. One can therefore anticipate that a carbonitrile-benzene derivative will achieve similar interaction with the dehydrogenase-binding pocket and display better inhibition due to the improved overall stabilisation. We are currently synthesising such carbonitrile-containing NAD^+ analogues to establish whether improved selectivity compared to that of the benzamide derivatives and potency compared to that of the thiophene series described here could be observed.

4. Experimental

4.1. Generalities

Chemicals were purchased from Sigma-Aldrich Chemical Company, Lancaster or ACROS. Solvents for extractions and chromatography were technical grade. Solvents used in reactions were freshly distilled from appropriated drying agents before use. All other reagents were recrystallised or

distilled as necessary. All reactions requiring anhydrous or inert conditions were carried out in oven-dried glassware under a positive atmosphere of argon. Solutions or liquids were introduced using oven dried syringes or cannula through rubber septa. All reactions were stirred magnetically using Teflon-coated stirs bars. In the cases requiring −78 °C cooling, the reactions were chilled with a dry ice/acetone bath. Removal of solvents was accomplished using a rotary evaporator at water aspirator pressure or under high vacuum (0.5 mm Hg). Analytical TLC was performed with Merck Silica gel 60 F_{254} plates. Visualisation was accomplished by UV-light (λ=254 nm) and/or staining with an anisaldehyde solution, followed by heating. Column chromatography were carried out using Fluorochem Silica gel 40−63 μm, 60 Å. HPLC monitoring was accomplished using a Supelcosil SAX1 (120 Å, 5 μm, 25 cm×4.6 mm) using a buffer KH_2PO_4 50 mM/MeOH 95/5, pH=3.5 at a flow rate of 1 mL/min. Purification of phosphate compounds were carried out as specified on BioRad AG®MP-1M resin (100–200 mesh, chloride form) or Amersham Biosciences DEAE Sepharose™ Fast Flow resin. ^{1}H, ^{13}C and 2D (H-COSY, HMQC) NMR spectra were all recorded on Brüker avance DPX 300 and Brüker avance DPX 500. Infrared spectra were recorded on a Perkin Elmer Spectrum RX 1 FT-IR System, using KBr discs. UV spectra were recorded using a Perkin Elmer Lambda 800 UV/Vis spectrometer. For known compounds, NMR data are compared to the one given in the literature. The names given between parenthesis use casual nomenclature.

4.2. Synthetic Procedures

5-*O*-*tert*-butyldimethylsilyl-2,3-*O*,*O*-isopropylidene-γ-ribonolactone 3.[14] *Method A*. γ-Ribonolactone (500 mg, 3.37 mmol) was suspended in 10 mL of acetone and 2,2-dimethoxypropane (0.83 mL, 13.49 mmol, 2 equiv.) and HCR-W2 H^+-Dowex resin (100 mg) were added. After stirring at room temperature for 4 h, the mixture was filtered and the resin rinsed with acetone. The filtrate was concentrated to give in a quantitative yield the intermediate as a white powder, which was used without further purification. δ ^{1}H ppm ($CDCl_3$, 500 MHz): 1.39 and 1.48 (s×2, 6H, $C(CH_3)_2$), 2.81 (br, 1H, 5-OH), 3.81 (bd, 1H, *J*=12.3 Hz, H-5a), 4.00 (bd, 1H, *J*=12.3 Hz, H-5b), 4.64 (t, 1H, *J*=1.9 Hz, H-4), 4.77 (d, 1H, *J*=5.6 Hz, H-3), 4.84 (d, 1H, *J*=5.7 Hz, H-2). δ ^{13}C ppm ($CDCl_3$, 75 MHz): 25.8 and 27.1 ($C(CH_3)_2$), 62.3 (C-5), 76.1, 78.7 (C-2,C-3), 83.2 (C-4), 113.5 ($C(CH_3)_2$), 175.5 (C=O-1). IR cm^{-1}(KBr): 3463 (OH), 2991, 2934 (CH), 1752 (C=O).

To a dry DCM solution (5 mL) of the partially protected lactone was added DMAP (41 mg, 0.34 mmol, 0.1 equiv.) and triethylamine (0.48 mL, 3.71 mmol, 1.1 equiv.). After cooling the solution to 0 °C, *tert*-butyldimethylsilyl chloride (560 mg, 3.71 mmol, 1.1 equiv.) was added. The mixture was stirred overnight under argon atmosphere. DCM (20 mL) was added to the solution and washed with a saturated solution of NH_4Cl then water. The resulting organic layer was dried over $MgSO_4$ and concentrated. The crude mixture is purified by silica gel column chromatography (PE/acetone 99/1) to give **3** as white crystals (688 mg, 68% over the two steps). **3**: δ ^{1}H ppm ($CDCl_3$, 300 MHz): 0.01 and 0.02 (s×2, 6H, $(CH_3)_2Si$), 0.84 (s, 9H, $(CH_3)_3CSi$), 1.35 and 1.42 (s×2, 6H, $C(CH_3)_2$), 3.74 (dd, 1H, *J*=11.3, 1.4 Hz, H-5a), 3.83 (dd, 1H, *J*=11.3, 2.1 Hz, H-5b), 4.55 (m, 1H, H-4), 4.65 and 4.67 (2×d, 2H, *J*=5.6, 5.6 Hz, H-2, H-3). δ ^{13}C ppm ($CDCl_3$, 75 MHz): −5.4 and −5.2 (($CH_3)_2Si$), 18.6 ($SiC(CH_3)_3$), 26.0, 26.1, 27.8 ($C(CH_3)_2$, $SiC(CH_3)_3$), 63.3 (C-5), 76.2, 78.8 (C-2, C-3), 82.6 (C-4), 113.4 ($C(CH_3)_2$), 174.5 (C=O-1). IR cm^{-1}(KBr): 2989, 2954, 2859 (CH), 1775 (C=O).

Method B. D-Ribose (5.0 g, 33.30 mmol) was suspended in acetone and 2,2-dimethoxypropane (8.2 mL, 66.60 mmol, 2 equiv.) followed by HCR-W2 H^+-Dowex resin, was added to the mixture. When the solution had become clear, the resin was filtered off and rinsed with acetone. After concentration, the crude yellow oil was dissolved in dry dichloromethane (15 mL) and DMAP (0.41 g, 3.33 mmol, 0.1 equiv.) and triethylamine (4.75 mL, 36.60 mmol, 1.1 equiv.) were added to the solution. After cooling the solution to 0 °C, *tert*butyldimethylsilyl chloride was added (5.52 g, 36.60 mmol, 1.1 equiv.) and the solution was stirred under argon atmosphere for 6 h. The mixture was then filtered over celite and washed with saturated aqueous NH_4Cl. The aqueous layer was extracted twice with dichloromethane. The combined organic layers were dried over $MgSO_4$, concentrated and used without purification. The crude mixture was dissolved in 300 mL of acetone and 10.53 g (66.60 mmol, 2 equiv.) of potassium permanganate are added. The solution was stirred at 50 °C for 6 h. The mixture was filtered over celite and concentrated. The same purification as described previously yielded **3** (4.00 g, 40% over the three steps).

2,2-Dimethyl-4-(3-cyano-thiophen-2-yl)-6-hydroxymethyl-tetrahydro-furo[3,4-*a*][1,3]dioxole 1a. (2-(2′,3′-*O*,*O*-isopropylidene-1′-deoxyribofuranosyl)thiophene-3-carbonitrile).

Intermediate 4a. 6-(*tert*-Butyl-dimethyl-silanoxymethyl)-2,2-dimethyl-4-(3-cyano-thiophen-2-yl)-tetrahydro-furo[3,4-*a*][1,3]dioxol-4-ol. (5-*O*-*tert*-butyldimethylsilyl-2,3-*O*,*O*-isopropylidene-1-(2-thiophene-3-carbonitrile)-α-D-ribofuranose).

n-Butyl lithium (2 M solution in pentane) (1.25 mL, 2.42 mmol, 1.5 equiv.) was added to a freshly distilled THF (20 mL) solution of diisopropylamine (0.35 mL, 2.42 mmol, 1.5 equiv.) at −78 °C under argon atmosphere. The solution was stirred at −78 °C for 5 min. Then thiophene-3-carbonitrile **2a** (0.226 mL, 2.42 mmol, 1.5 equiv.) was added and the solution was stirred at −78 °C for 20 min. To this mixture was added by cannula a solution of **3** (500 mg, 1.66 mmol, 1 equiv.) in dry THF (20 mL). The solution was stirred at −78 °C for 30 min then the reaction was quenched at −60 °C by addition of ether (30 mL) and a saturated solution of NH_4Cl (30 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ then water. The resulting organic layers were gathered, dried on $MgSO_4$ and concentrated. For analysis purposes, it was purified by silica gel chromatography column (hexane/EtOAc 95/5 to 1/1) to obtain **4a** (468 mg, 69%) as a light yellow oil. However, this compound is readily decomposed upon storage. **4a**: δ ^{1}H ppm ($CDCl_3$, 300 MHz): 0.17, 0.18 (s×2, 3H×2, $(CH_3)_2Si$), 0.96 (s, 9H, $(CH_3)_3CSi$), 1.25, 1.48 (s×2, 3H×2, $C(CH_3)_2$), 3.84 (dd, 1H,

J=2.1, 11.2 Hz, H-5a), 3.90 (dd, 1H, J=2.2, 11.2 Hz, H-5b), 4.56 (m, 1H, H-4), 4.71 (d, 1H, J=5.7 Hz, H-2), 4.90 (dd, 1H, J=1.2, 5.7 Hz, H-3), 7.22 (d, 1H, J=5.2 Hz, H_{Th}-4), 7.36 (d, 1H, J=5.2 Hz, H_{Th}-5). δ ^{13}C ppm ($CDCl_3$, 75 MHz): −5.2 (($CH_3)_2$Si), 18.7 (($CH_3)_3C$Si), 25.0, 26.2, 26.4 (C($CH_3)_2$, ($CH_3)_3$CSi), 64.9 (C-5), 82.0 (C-3), 87.1 (C-4), 89.0 (C-2), 106.1 (C-1), 109.5, 113.7, 115.6 (C_{Th}-3, CN, $C(CH_3)_2$), 126.3 (C_{Th}-4), 130.3 (C_{Th}-5), 152.2 (C_{Th}-2).

Intermediate 5a. Acetic acid 6-(*tert*-butyl-dimethyl-silanoxymethyl)-2,2-dimethyl-4-(3-cyano-thiophen-2-yl)-tetrahydro-furo[3,4-*a*][1,3]dioxol-4-yl ester. (1-*O*-acetyl-5-*O*-*tert*-butyldimethylsilyl-2,3-*O*,*O*-isopropylidene-1-(2-thiophene-3-carbonitrile)-α-D-ribofuranose).

n-Butyl lithium (2 M solution in pentane) (1.25 mL, 2.42 mmol, 1.5 equiv.) was added to a freshly distilled THF (20 mL) solution of diisopropylamine (0.35 mL, 2.42 mmol, 1.5 equiv.) at −78 °C under argon atmosphere. The solution was stirred at −78 °C for 5 min. Then thiophene-3-carbonitrile **2a** (0.226 mL, 2.42 mmol, 1.5 equiv.) was added and the solution was stirred at −78 °C for 20 min. To this mixture was added by cannula a solution of **3** (500 mg, 1.66 mmol, 1 equiv.) in dry THF (20 mL). The solution was stirred at −78 °C for 30 min then warmed to −70 °C/−60 °C and acetic anhydride (0.78 mL, 8.28 mmol, 5 equiv.) was added. After stirring another 30 min at −60 °C, the reaction was quenched at −60 °C by addition of ether (30 mL) and a saturated solution of NH_4Cl (30 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ then water. The combined aqueous layers were washed with ether and the resulting organic layers were gathered, dried on $MgSO_4$ and concentrated. This crude mixture was nearly pure and used without purification for the next step. For analysis purposes, it was purified by silica gel chromatography column (PE/Acetone 9/1) to obtain **5a** (713 mg, 95%) as a light yellow oil However this compound is readily decomposed upon storage. **5a**: δ ^{1}H ppm ($CDCl_3$, 300 MHz): 0.06 and 0.07 (s×2, 6H, ($CH_3)_2$Si)), 0.86 (s, 9H, ($CH_3)_3$CSi), 1.39, 1.73 (s×2, 3H×2, C($CH_3)_2$), 2.25 (s, 3H, OAc), 3.83 (dd, 1H, J=11.3, 2.6 Hz, H-5a), 3.91 (dd, 1H, J=11.3, 3.2 Hz, H-5b), 4.49 (m, 1H, H-4), 4.61 (d, 1H, J=6.4 Hz, H-2), 4.83 (dd, 1H, J=6.4, 2.1 Hz, H-3), 7.14 (d, 1H, J=5.3 Hz, H_{Th}-4), 7.33 (d, 1H, J=5.3 Hz, H_{Th}-5). δ ^{13}C ppm ($CDCl_3$, 75 MHz): −5.2 and −4.9 (($CH_3)_2$Si), 18.6 (($CH_3)_3C$Si), 21.8 (CH_3 Ac), 26.1 (), 26.2 (($CH_3)_3$CSi), 63.0 (C-5), 81.1 (C-2), 84.9 (C-4), 88.7 (C-3), 103.7 (C-1), 106.8, 114.5, 116.3 (C_{Th}-3, CN, $C(CH_3)_2$), 125.8 (C_{Th}-5), 130.4 (C_{Th}-4), 153.8 (C_{Th}-2), 168.8 (OAc). IR (KBr) cm^{-1}: 2932, 2858 (CH), 2232 (CN), 1763 (C=O). MS (LSIMS) M-OAc: 394.

The crude mixture of **5a** (1.19 mmol) was dissolved in dry dichloromethane (10 mL) with molecular sieves, under argon atmosphere. Triethylsilane (0.95 mL, 5.97 mmol, 5 equiv.) and freshly distilled trimethylsilyl trifluoromethanesulfonate (0.54 mL, 2.98 mmol, 2.5 equiv.) were added to the solution at 0 °C. The reaction was then warmed up to room temperature and stirred for 30 min. No remaining starting material was detected on TLC, triethylamine (2 mL) were added to the solution and then tetrabutylammonium fluoride (1 M solution in THF) (2.4 mL, 2.38 mmol, 2 equiv.). After 4 h, the mixture was quenched by a saturated solution of NH_4Cl and extracted with dichloromethane. The organic layer was washed with water, dried on $MgSO_4$ and concentrated. After purification on silica gel chromatography column, **1aα** (74 mg, 29%) and **1aβ** (54 mg, 21%) were isolated. **1aβ**: δ ^{1}H ppm ($CDCl_3$, 300 MHz): 1.37, 1.64 (s×2, 3H×2, C($CH_3)_2$), 2.23 (m, 1H, OH-5′), 3.85 (ddd, 1H, J=12.4, 3.6, 8.5 Hz, H-5′a), 4.00 (ddd, 1H, J=12.4, 2.8, 4.45 Hz, H-5′b), 4.25 (m, 1H, H-4′), 4.63 (m, 1H, H-2′), 4.87 (dd, 1H, J=3.6, 6.6 Hz, H-3′), 5.20 (d, 1H, J=5.7 Hz, H-1′), 7.23 (d, 1H, J=5.3 Hz, H_{Th}-4), 7.33 (d, 1H, J=5.3 Hz, H_{Th}-5). δ ^{13}C ppm ($CDCl_3$, 75 MHz): 25.7, 27.9 (C($CH_3)_2$), 62.9 (C-5′), 81.8, 81.8 (C-1′, C-3′), 85.5 (C-4′), 87.4 (C-2′), 108.1, 115.4, 115.8 (C_{Th}-3, CN, $C(CH_3)_2$), 126.2 (C_{Th}-5), 129.8 (C_{Th}-4), 153.1 (C_{Th}-2). IR (KBr) cm^{-1}: 3447 (OH), 2926 (CH), 2229 (CN). MS (LSIMS) M+H calculated: 282.0800; measured: 282.0795. **1aα**: δ ^{1}H ppm ($CDCl_3$, 300 MHz): 1.34, 1.49 (s×2, 3H×2, C($CH_3)_2$), 1.91 (m, 1H, OH-5′), 3.85 (m, 2H, H-5′a and H-5′b), 4.34 (m, 1H, H-4′), 4.88 (dd, 1H, J=0.9, 5.9 Hz, H-3′), 4.97 (dd, 1H, J=4.0, 5.9 Hz, H-2′), 5.65 (d, 1H, J=4.0 Hz, H-1′), 7.19 (d, 1H, J=5.3 Hz, H_{Th}-4), 7.38 (d, 1H, J=5.3 Hz, H_{Th}-5). δ ^{13}C ppm ($CDCl_3$, 75 MHz): 25.1, 26.5 (C($CH_3)_2$), 63.2 (C-5′), 79.3 (C-1′), 82.1 (C-2′), 83.3 (C-3′), 85.1 (C-4′), 108.1, 113.7, 115.1 (C_{Th}-3, CN, $C(CH_3)_2$), 127.6 (C_{Th}-5), 128.0 (C_{Th}-4), 151.5 (C_{Th}-2). IR (KBr) cm^{-1}: 3436 (OH), 2924 (CH), 2230 (CN). MS (LSIMS) M+H calculated: 282.0800; measured: 282.0806.

2,2-Dimethyl-4-(2-cyano-thiophen-5-yl)-6-hydroxy-methyl-tetradro-furo[3,4-*a*][1,3]dioxole 1b. (5-(2′, 3′-*O*,*O*-isopropylidene-1′-deoxyribofuranosyl)thiophene-2-carbonitrile).

Intermediate 4b. 6-(*tert*-Butyl-dimethyl-silanoxymethyl)-2,2-dimethyl-4-(2-cyanothiophen-5-yl)-tetrahydro-furo[3,4-*a*][1,3]dioxol-4-ol. (5-*O*-*tert*-butyldimethylsilyl-2,3-*O*,*O*-isopropylidene-1-(5-thiophene-2-carbonitrile)-α-D-ribofuranose).

The same procedure as the one used to prepare **4a** was used to prepare **4b** for analysis purposes. However, the compound is readily decomposed upon storage. **4b** δ ^{1}H ppm ($CDCl_3$, 300 MHz): 0.18, 0.19 (s×2, 3H×2, ($CH_3)_2$Si), 0.96 (s, 9H, ($CH_3)_3$CSi), 1.30, 1.56 (s×2, 3H×2, C($CH_3)_2$), 3.83 (dd, 1H, J=2.1, 11.3 Hz, H-5a), 3.87 (dd, 1H, J=2.0, 11.3 Hz, H-5b), 4.46 (m, 1H, H-4), 4.59 (d, 1H, J=5.7 Hz, H-2), 4.90 (dd, 1H, J=4.6, 5.7 Hz, H-3), 7.24 (d, 1H, J=4.1 Hz, H_{Th}-4), 7.67 (d, 1H, J=4.1 Hz, H_{Th}-3). δ ^{13}C ppm ($CDCl_3$, 75 MHz): −5.3(($CH_3)_2$Si), 18.7 (($CH_3)_3C$Si), 25.2, 26.2, 26.8 (C($CH_3)_2$, ($CH_3)_3$CSi), 64.9 (C-5), 82.2 (C-2), 86.8 (C-4), 88.9 (C-3), 105.8, 110.2, 113.6, 115.0 (C-1, C_{Th}-2, CN, $C(CH_3)_2$), 126,9 (C_{Th}-4), 137.3 (C_{Th}-3), 150.5 (C_{Th}-5). IR (KBr) cm^{-1}: 3328 (OH), 2933, 2859 (CH), 2221.1 (CN).

Intermediate 5b. Acetic acid 6-(*tert*-butyl-dimethyl-silanoxymethyl)-2,2-dimethyl-4-(3-cyano-thiophen-5-yl)-tetrahydro-furo[3,4-*a*][1,3]dioxol-4-yl ester. (1-*O*-acetyl-5-*O*-*tert*-butyldimethylsilyl-2,3-*O*,*O*-isopropylidene-1-(5-thiophene-2-carbonitrile)-α-D-ribofuranose).

The same procedure as the one used to prepare **5a** was used to prepare **5b**. Purification for analysis purposes on silica gel

column (PE/Acetone 95/5) afforded pure **5b** as a powder (95%). However the compound is readily decomposed upon storage. **5b**: δ ^{1}H ppm ($CDCl_3$, 300 MHz): 0.05 and 0.06 (s×2, 3H×2, $(CH_3)_2Si$), 0.85 (s, 9H, $(CH_3)_3CSi$),1.39, 1.69 (s×2, 3H×2, $C(CH_3)_2$), 2.18 (s, 3H, OAc), 3.81 (dd, 1H, J=2.5, 11.3 Hz, H-5a), 3.90 (dd, 1H, J=2.8, 11.3 Hz, H-5b), 4.48 (m, 1H, H-4), 4.61 (d, 1H, J=6.4 Hz, H-2), 4.83 (dd, 1H, H-3, J=2.1, 6.4 Hz, H-2), 7.10 (d, 1H, J=3.9 Hz, H_{Th}-4), 7.47 (d, 1H, J=3.9 Hz, H_{Th}-3). δ ^{13}C ppm ($CDCl_3$, 75 MHz): −5.2, −5.0 ($(CH_3)_2Si$), 18.6 ($(CH_3)_3CSi$), 23.0 (OAc), 25.9, 26.2 ($C(CH_3)_2$, $(CH_3)_3CSi$), 63.1 (C-5), 81.0 (C-2), 85.0 (C-4), 88.3 (C-3), 124.9 (C_{Th}-4), 137.6 (C_{Th}-3).

The same procedure as the described for **1a** was used for the synthesis of **1b** and afford 27% of α isomer and 20% of β isomer. **1bβ**: δ ^{1}H ppm ($CDCl_3$, 300 MHz): 1.37, 1.60 (s×2, 3H×2, $C(CH_3)_2$), 3.77 (dd, 1H, J=4.1, 12.1 Hz, H-5′a), 3.89 (dd, 1H, J=3.3, 12.1 Hz, H-5′b), 4.22 (m, 1H, H-4′), 4.59 (dd, 1H, J=5.3, 6.6 Hz, H-2′), 4.79 (dd, 1H, J=3.7, 6.6 Hz, H-3′), 5.10 (d, 1H, J=5.2 Hz, H-1′), 7.07 (d, 1H, J=3.8 Hz, H_{Th}-4), 7.54 (d, 1H, J=3.8 Hz, H_{Th}-3). δ ^{13}C ppm ($CDCl_3$, 75 MHz): 25.8, 27.8 ($C(CH_3)_2$), 63.0 (C-5′), 82.1, 82.9 (C-1′, C-3′), 85.5 (C-4′), 87.2 (C-2′), 109.3, 114.5, 115.7 (C_{Th}-2, CN, $C(CH_3)_2$), 124.6 (C_{Th}-4), 138.1 (C_{Th}-3), 151.7 (C_{Th}-5). IR (KBr) cm^{-1}: 3445 (OH), 2925 (CH), 2228 (CN). MS (LSIMS) M+H calculated: 282.0800; measured: 282.0807. **1bα**: δ ^{1}H ppm ($CDCl_3$, 300 MHz): 1.34, 1.53 (s×2, 3H×2, $C(CH_3)_2$), 1.86 (bs, 1H, OH-5′), 3.82 (m, 2H, H-5′a and H-5′b), 4.30 (m, 1H, H-4′), 4.83 (dd, 1H, J=3.9, 5.9 Hz, H-2′), 4.88 (dd, 1H, J=1.0, 5.9 Hz, H-3′), 5.43 (d, 1H, J=3.9 Hz, H-1′), 7.07 (d, 1H, J=3.8 Hz, H_{Th}-4), 7.52 (d, 1H, J=3.8 Hz, H_{Th}-3). δ ^{13}C ppm ($CDCl_3$, 75 MHz): 25.2, 26.5 ($C(CH_3)_2$), 63.5 (C-5′), 80.1 (C-1′), 82.5 (C-2′), 83.3 (C-3′), 84.9 (C-4′), 110.7, 113.8 (C_{Th}-2, CN, $C(CH_3)_2$), 126.2 (C_{Th}-4), 136.8 (C_{Th}-3), 147.8 (C_{Th}-5). IR (KBr) cm^{-1}: 3467 (OH), 2925 (CH), 2226 (CN). MS (LSIMS) M+H calculated: 282.0800; measured: 282.0785.

β-2(3-cyanothiophene) adenine dinucleotide 7aβ

Intermediate 6aβ. Phosphoric acid mono-[5(*R*)-(3-cyano-thiophen-2-yl)-3,4-dihydroxy-tetrahydro-furan-2-yl methyl] ester. (2-β-D-[1′-(5′-phosphateribofuranosyl)] thiophene-3-carbonitrile).

Compound **1aβ** (32 mg, 0.114 mmol) was solubilised in triethyl phosphate (1.2 mL) and heated at 50 °C. Then after cooling down at 0 °C, phosphorus oxychloride (32 μL, 0.342 mmol, 3 equiv.) was added. The mixture was stirred for 2 days at room temperature and monitored by anion exchange HPLC (Supelcosil SAX1, buffer KH_2PO_4 50 mM/MeOH 95/5). Another 32 μL of $POCl_3$ were added if needed to complete the reaction. The reaction was quenched at 0 °C by addition of ice-cold water and stirred for 1 h. The mixture was extracted with diethyl ether (20 mL×3) and the aqueous layer was freeze-dried. The crude mixture was purified on AG MP1 resin with a gradient of 0–150 mM TFA to give **6aβ** (10 mg, 25%). **6aβ**: δ ^{1}H ppm (D_2O, 500 MHz): 3.95 (m, 2H, H-5′a, H-5′b), 4.10–4.17 (m, 3H, H-2′, H-3′, H-4′), 5.17 (d, 1H, J=7.1 Hz, H-1′), 7.18 (d, 1H, J=5.0 Hz, H-4), 7.43 (d, 1H, J=5.0 Hz, H-5). δ ^{13}C ppm (D_2O, 125 MHz): 65.9 (C-5′), 72.1, 78.4 (C-2′, C-3′), 79.4 (C-1′), 84.4 (d, $J_{C-C-O-P}$=8.5 Hz, C-4′), 108.1, 115.7 (C-3, CN), 128.1 (C-5), 129.5 (C-4), 154.6 (C-2). δ ^{31}P ppm (D_2O, 121 MHz): 1.3 (s). MS (ESI-) M−H: 320.

Compound **6aβ** (11 mg, 34.3 μmol) was dissolved in a 0.2 M solution of manganese chloride in formamide left on molecular sieves during several days (0.5 mL). $MgSO_4$ (8 mg, 68.5 μmol, 2 equiv.) and adenosine 5′-monophosphomorpholidate·4-morpholine-*N*,*N*′-dicyclohexylcarboxamidine salt (48.6 mg, 68.5 μmol, 2 equiv.) were added to this solution. The mixture was sonicated and left to react for 4 days after which no evolution was observed by HPLC SAX monitoring. 20 μL of water were added to quench the reaction. The mixture was then purified on a DEAE sepharose resin column eluted with a gradient of triethylammonium formate 20 to 250 mM to give **7aβ** with 39% yield. **7aβ**: δ ^{1}H ppm (D_2O, 500 MHz): 3.60–4.45 (m, protons of the sugars), 4.73 (d, 1H, J=6.04 Hz, H_{Th}-1′), 5.67 (d, 1H, J=4.0 Hz, H_{Ad}-1′), 6.68 (d, 1H, J=4.9 Hz, H_{Th}-4), 6.90 (d, 1H, J=4.7 Hz, H_{Th}-5), 7.70 (Ad). δ ^{31}P ppm (D_2O, 121 MHz): −9.53 (m). λ_{max} (H_2O)=253 nm. MS (ES-) M−H calculated: 649.0525, measured: 649.0528.

β-5(2-cyanothiophene) adenine dinucleotide 7bβ

Intermediate 6bβ: phosphoric acid mono-[5(*R*)-(2-cyano-thiophen-5-yl)-3,4-dihydroxy-tetrahydro-furan-2-yl methyl] ester. (5-β-D-[1′-(5′-phosphateribofuranosyl)] thiophene-2-carbonitrile).

The same procedure as described for **6aβ** was used for the synthesis of **6bβ** (25%). **6bβ**: δ ^{1}H ppm (D_2O, 500 MHz): 4.20 (m, 2H, H-5′a, H-5′b), 4.29–4.44 (m, 3H, H-2′, H-3′, H-4′), 5.25 (d, 1H, J=7.1 Hz, H-1′), 7.36 (d, 1H, J=3.7 Hz, H_{Th}-4), 7.85 (d, 1H, J=3.8 Hz, H_{Th}-3). δ ^{13}C ppm (D_2O, 75 MHz): 67.3 (d, J_{C-O-P}=5.0 Hz, C-5′), 73.5, 79.5 (C-3′, C-2′) 81.3 (C-1′), 85.6 (d, $J_{C-C-O-P}$=8.7 Hz, C-4′), 109.8, 117.0 (C_{Th}-2, CN), 127.8 (C_{Th}-4), 141.2 (C_{Th}-3), 153.4 (C_{Th}-5). δ ^{31}P ppm (D_2O, 121 MHz): 1.3 (s). MS (ESI-) M−H: 320. The same procedure as described for **7aβ** was used for the synthesis of **7bβ** (42%). **7bβ**: δ ^{1}H ppm (D_2O, 500 MHz): 3.7–3.9 (m, H-5′ ×4), 4.0–4.4 (m, protons of the sugars), 5.75 (d, J=3.6 Hz, H_{Ad}-1′), 6.74 (H_{Th}-4), 7.22 (H_{Th}-3). δ ^{13}C ppm (D_2O, 75 MHz): 67.9, 68.5, 69.4, 72.9, 74.1, 77.1, 80.3, 82.4, 86.0, 86.5, 90.3, 109.9, 117.7, 128.1, 141.9, 152.4, 155.0. δ ^{31}P ppm (D_2O, 121 MHz): −10.0 (m). λ_{max} (H_2O)=260 nm. MS (ES-) M−H calculated: 649.0525, measured: 649.0530.

α-5(2-cyanothiophene) adenine dinucleotide 7bα

Intermediate 6bα: phosphoric acid mono-[5(*S*)-(2-cyano-thiophen-5-yl)-3,4-dihydroxy-tetrahydro-furan-2-yl methyl] ester. (5-α-D-[1′-(5′-phosphateribofuranosyl)] thiophene-2-carbonitrile).

The same procedure as described for **6aβ** was used for the synthesis of **6bα** (15%). **6bα**: δ ^{1}H ppm (D_2O, 300 MHz): 3.70–4.39 (m, 5H, H-2′, H-3′, H-4′, H-5′a, H-5′b), 5.36 (broad s, 1H, H-1′), 7.02 (d, 1H, J=3.6 Hz, H_{Th}-4), 7.59 (d, 1H, J=3.6 Hz, H_{Th}-3). δ ^{13}C ppm (D_2O, 75 MHz): 65.4 (d, J_{C-O-P}=4.83 Hz, C-5′), 72.4, 73.7(C-2′, C-3′), 79.5 (C-1′), 80.9 (d, $J_{C-C-O-P}$=7.9 Hz, C-4′), 108.7, 115.7 (C_{Th}-2,

CN), 126.8 (C_{Th}-4), 139.1 (C_{Th}-3), 149.0 (C_{Th}-5). δ ^{31}P ppm (D_2O, 121.45 MHz): 1.7 (s). MS (ESI-) M−H: 320.

The same procedure as described for **7aβ** was used for the synthesis of **7bα** (30%). **7bα**: δ ^{1}H ppm (D_2O, 500 MHz): 3.7–4.5 (m, protons of the sugars), 5.05 (s, 1H, H_{Th}-1′), 5.90 (d, 1H, J=5.05 Hz, H_{Ad}-1′), 6.71 (d, 1H, H_{Th}-4), 7.32 (d, 1H, J=3.0 Hz, H_{Th}-3), 8.05 (s, H_{Ad}-2), 8.34 (s, H_{Ad}-8). δ ^{31}P ppm (D_2O, 121 MHz): −9.79 (m). λ_{max} (H_2O)=261 nm. MS (ES-) M−H calculated: 649.0525, measured: 649.0527.

Acknowledgements

We thank the MRC for funding (Grant number G0001004) and the EPSRC National Mass Spectrometry Service Centre, Swansea for mass spectra. We also thank Dr S.D. Bell from the Hutchinson/MRC Research Centre, Cancer Cell Unit in Cambridge for supplying Sir2 samples.

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