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Synthesis of thiazole-4-carboxamide-adenine difluoromethylenediphosphonates substituted with fluorine at C-2′ of the adenosine

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ABSTRACT

Synthesis of an analogue **3** of thiazole-4-carboxamide adenine-dinucleotide (TAD) in which the β-oxygen atom of the pyrophosphate bridge is replaced by a difluoromethylene group has been achieved. Likewise, 2′-deoxy-2′-fluoroadenosine containing analogues of TAD (**4**) and its difluoromethylenediphosphonate congener (**5**) have been synthesized. Adenosine 5′-difluoromethylenediphosphonate (**8**) was prepared from 5′-*O*-tosyladenosine (**6**) and tris(tetra-*n*-butylammonium)difluoromethylenediphosphonate (**7**) by a modified procedure of Poulter's.[2] Compound **8** was converted into the 2′,3′-cyclic carbonate **9** by treatment with triethyl orthoformate. Treatment of **9** with 2′,3′-*O*-isopropylidenetiazofurin (**10**) in pyridine in the presence of DCC gave a mixture of dinucleotide **11** and the isopropylidene-protected diadenosine tetraphosphonate **12**. After deprotection of **11**, the desired β-difluoromethylene TAD (**3**) was separated by HPLC as the minor product. The diadenosine tetraphosphonate **12**, an analogue of Ap_4A, was obtained as the major component. Alternatively, 2′,3′-*O*-isopropylidenetiazofurin (**10**) was tosylated, and the product **13** was further converted into the corresponding difluoromethylenediphosphonate **14** by coupling with **7**. DCC-catalyzed coupling of **14** with 2′-deoxy-2′-fluoroadenosine (**15**) followed by deisopropylidenation afforded the analogue **5**. Again the corresponding tetraphosphonate analogue of tiazofurin **17** was the predominant product. Dinucleotide **4** was obtained by coupling of the carbonyldiimidazole-activated tiazofurin 5′-monophosphate with 2′-deoxy-2′-fluoroadenosine 5′-monophosphate. 2′-Deoxy-2′-fluoroadenosine (**15**) was prepared efficiently from the known N^6-benzoyl-3′-*O*-tetrahydropyranyladenosine (**18**), which was converted into 3′-*O*-tetrahydropyranyl-2′-*O*-triflyl-5′-*O*-tritylադenosine (**20**) by tritylation and triflation. Treatment of **20** with sodium acetate in hexamethylphosphoric triamide, followed by deacetylation afforded 9-(3-*O*-tetrahydropyranyl-5-*O*-trityl-β-D-arabinofuranosyl)-N^6-benzoyladenine (**22**), which was then treated with DAST. After deprotection of the product, **15** was obtained in good yield.

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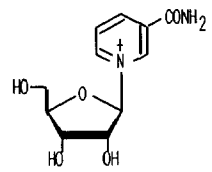
† Part 2 of the series NAD Analogues. For Part 1, see ref. 1.

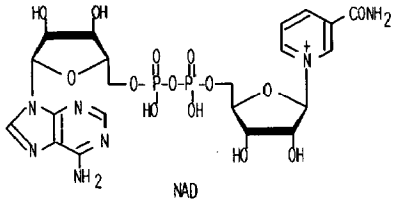
INTRODUCTION

Thiazole-4-carboxamide adenine dinucleotide (**1**, TAD, Fig. 1), is the active metabolite of the oncolytic C-nucleoside, 2-(β-D-ribofuranosyl)thiazole-4-carboxamide (Tiazofurin, TF), which was found to be a potent inhibitor of IMP-dehydrogenase.[3–14] Tiazofurin is phosphorylated by adenosine kinase and/or 5′-nucleotidase to the 5′-monophosphate and then coupled with adenosine 5′-monophosphate (AMP) by NAD-pyrophosphorylase to give TAD[15,16]. TAD is an analogue of nicotinamide adenine dinucleotide (NAD) in which the nicotinamide riboside residue is replaced by tiazofurin. It mimics NAD but cannot function as the coenzyme.

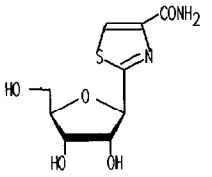
Resistance to tiazofurin is associated with increased degradation of TAD by a phosphodiesterase ("TADase")[17–19]. Recently Marquez et al.[20] synthesized the



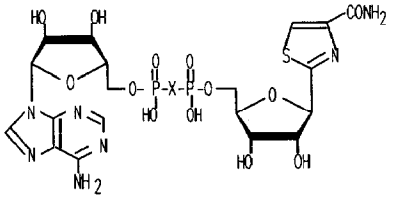
"Nicotinamide riboside"



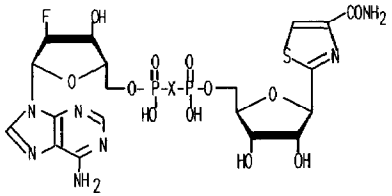
NAD



Tiazofurin



1, X = O; TAD
2, X = CH_2; β-methylene TAD
3, X = CF_2; β-difluoromethylene TAD



4 X = O
5 X = CF_2

Fig. 1. Tiazofurin analogues.

phosphonate analogue of TAD (**2**, β-methylene TAD) replacing the pyrophosphate oxygen with a methylene group. As expected, **2** was found to be resistant to metabolic cleavage by TADase and showed significant cytotoxicity against tiazofurin-resistance cell lines.

In this paper we report the synthesis of β-difluoromethylene-TAD (**3**), which should be equally resistant to TADase as **2**, but may better mimic the natural pyrophosphate **1**. An individual fluorine may serve as isosteric replacement for hydrogen or hydroxyl group, while a $-CF_2-$ group can function as an isopolar and isosteric substitute for oxygen[21]. The physical properties of a range of α-γ-bridged phosphonate analogues showed[22] that an increasing correspondence to those of ATP is found to be in the sequence of $CH_2 < CHF{=}CCl_2 < CF_2{=}NH < O$.

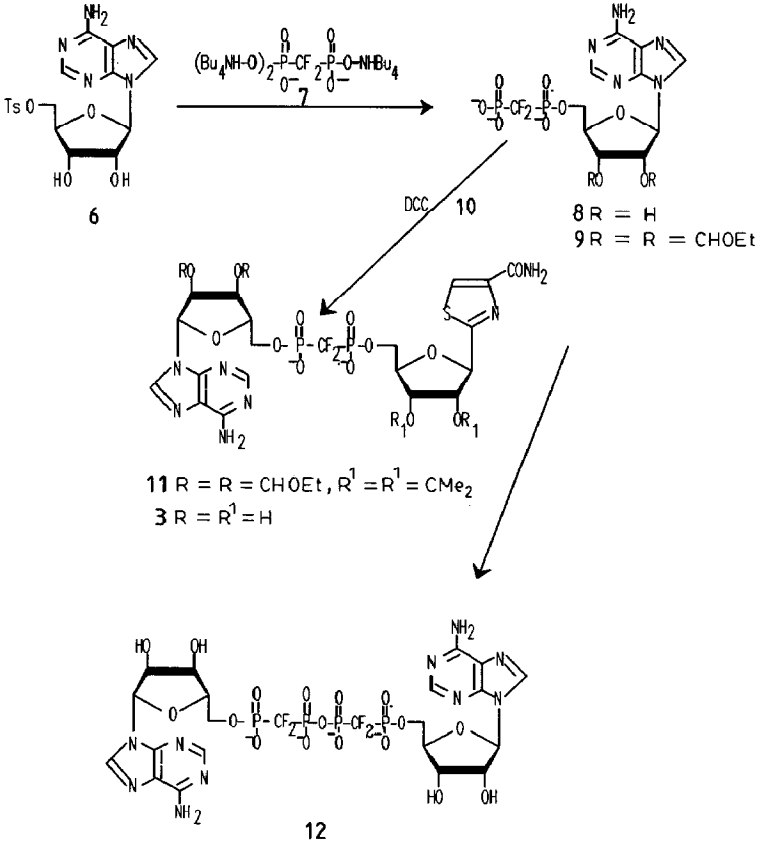
Clinical studies with TF revealed that this C-nucleoside exerts a variety of toxic side effects, partially due to inhibition of other cellular dehydrogenases[23]. Some dehydrogenases depend on NAD as the cofactor, others use NAD(P). The latter arises from NAD, in the cytosol of most cells, by phosphorylation of the 2′-hydroxy group of the adenosine moiety. If a "false" cofactor, such as TAD, is phosphorylated in a similar manner, then the product may inhibit the NAD(P)-dependent enzymes. It is therefore of interest to synthesize tiazofurin–adenine dinucleotides substituted with fluorine at C-2′ of the ribosyl group of adenosine (**4** and **5**. Fig. 1). Compounds **4** and **5** cannot be converted into the corresponding NAD(P) analogues and consequently are expected to be less toxic than TAD itself, since analogues **4** and **5** should be harmless toward NAD(P)-dependent cellular enzymes. These TAD analogues (**3**, **4**, and **5**), being more hydrophobic than their corresponding hydroxy analogues, may better fit in the hydrophobic binding-pocket of dehydrogenases.

It is well documented by X-ray structure determination of various dehydrogenase–NAD complexes that the 2′- and 3′-hydroxy groups, and the furanose ring oxygen of the cofactor, are fixed by hydrogen bonds to appropriate amino acid residues, when NAD binds to dehydrogenases[24–26]. Fluorine can form even stronger hydrogen-bonds than oxygen, acting as a proton acceptor.

Fluorine is not a sterically demanding substituent. It has a small van der Waals radius (1.35 Å) similar to that of hydrogen (1.29 Å). On the other hand, the carbon–fluorine bond length (1.39 Å) closely resembles that of the carbon–oxygen bond length (1.43 Å). Thus, fluorine should mimic a hydroxyl group well in terms of size and polarity.

RESULTS AND DISCUSSION

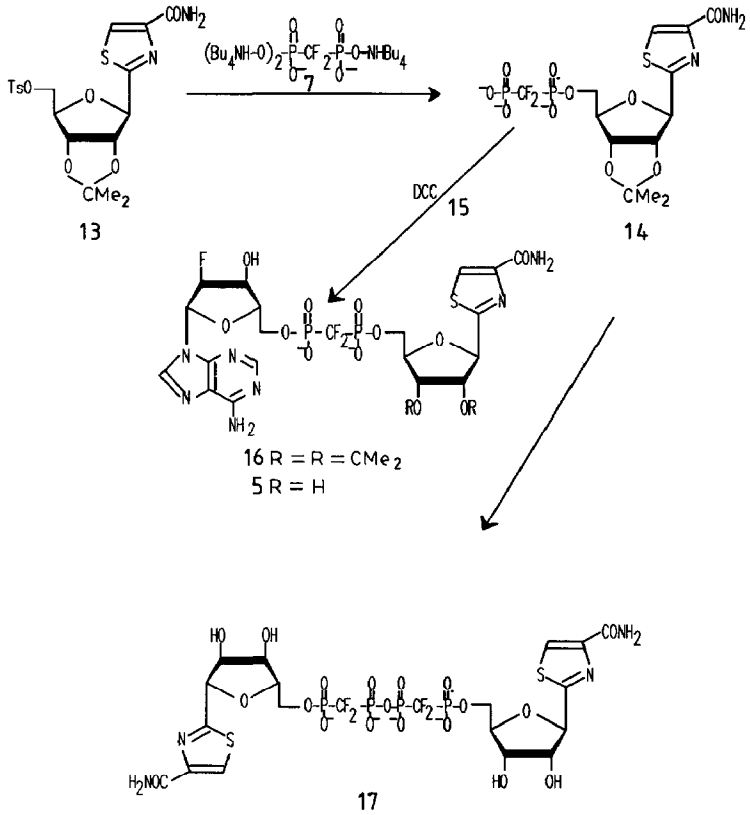
The synthesis of adenosine 5′-difluoromethylenediphosphonate (**8**, Scheme 1) according to Poulter's procedure[2] required 4 mmols of tris(tetra-*n*-butylammonium)hydrogen difluoromethylenediphosphonate (**7**) for 1 mmol of the 5′-*O*-tosyladenosine and 66 h in acetonitrile. In our hands, because of low solubility of the diphosphonate salt **7** (even when an ultrasonic bath was used) the reaction



Scheme 1.

proceeded even more slowly, giving a lower yield than reported. When dimethyl sulfoxide was used instead of acetonitrile, the reaction was complete in 1 h, and the desired product was obtained in 65% yield. The dinucleotide **8** was then protected with triethyl orthoformate to give **9**, which, without purification, was coupled with 2′,3′-*O*-isopropylidenetiazofurin (**10**) in the presence of DCC. The crude mixture was treated with Dowex 50W-X8 (H^+ form) to remove the isopropylidene and orthoformate groups. Purification on a preparative HPLC column gave the desired dinucleotide **3** in 15.1% yield and the known pyrophosphate derivative **12** in 57% yield. The pyrophosphate **12**, containing two adenosine 5′-difluoromethylenediphosphonate residue, was earlier synthesized by treatment of adenosine 5′-difluoromethylenediphosphonate with DCC in pyridine[27]. The compound is of biological interest as it is related to diadenosine tetraphosphate (Ap_4A), which is known to function as an intracellular "alarmone" in connection with environmental stress[28]. The tetraphosphate also affects cell proliferation, platelet aggregation, and ADP-ribosylation processes.

In the coupling reaction of **9** with **10**, the formation of pyrophosphate **12** was always preferred to that of dinucleotide **3** regardless the amount of DCC (10–15 equivs) used. Surprisingly, in a similar reaction of adenosine 5′-methylenediphosphonate with **10**, the formation of the corresponding diadenosine tetraphosphate was not reported[20]. This is probably due to the strong electron-withdrawing effect of the $-CF_2-$ group of **9**, which makes the nucleotide more acidic than adenosine 5′-methylenediphosphonate. Thus, after initial formation of the betaine-type intermediate of **9** with DCC, the formal negative charge at the phosphorus oxygen is still preserved, rendering nucleophilic attack of nucleoside **10** more difficult than in the case of methylene diphosphonate derivative. On the other hand, the difluoromethylenediphosphonate derivative, more acidic than methylenediphosphonate, can protonate the nitrogen atom of intermediate of **9** with DCC more effectively, facilitating the departure of *N,N′*-dicyclohexylurea with the formation of a pyrophosphate bond. The lower yield of **3** than that reported by Marquez[20] for the preparation of **2** may partly be due to some cleavage of the $P-CF_2$ bond, which is weaker than the $P-CH_2$ linkage[29]. The



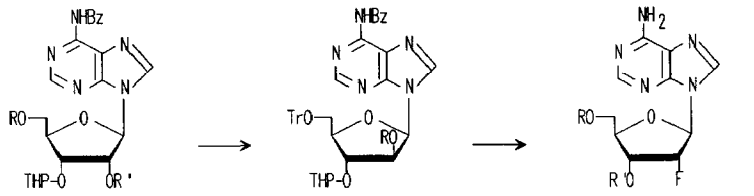
Scheme 2.

progress of the coupling reaction of **9** with **10** could be monitored by ^{31}P NMR. The ^{31}P NMR spectrum of derivative **9** contains 12 resonance signals (ABX_2), but after coupling is completed, six resonances almost vanished to give a quasi double triplet ($AA'X_2$) because of the identical chemical shifts of the two phosphorus atoms of the product.

Similarly, the β-difluoromethylene TAD (**5**) containing 2′-deoxy-2′-fluoroadenosine (**15**) was prepared (Scheme 2). Thus, the 2′,3′-*O*-isopropylidenetiazofurin (**10**) was treated with TsCl to give nucleoside **13**, which, by reaction with tris (tetra-*n*-butylammonium) hydrogen difluoromethylenediphosphonate, afforded the protected tiazofurin 5′-difluoromethylenediphosphonate **14**. Coupling of **14** with 2′-deoxy-2′-fluoroadenosine (**15**) gave a mixture of the desired dinucleotide **16** and the isorpopylidene-protected pyrophosphate derivative of **17**. No reaction at the 3′-hydroxy group of **15** occurred because of the low nucleophilicity of the 3′-hydroxy function conferred by the presence of the neighboring fluorine atom. Treatment of the mixture with aqueous trifluoroacetic acid, followed by preparative HPLC separation, afforded the desired TAD analogue **5** in 4.5% yield and the ditiazofurin tetraphosphonate analogue **17** as the major product (53% yield). The reactions of DCC-catalyzed coupling of **9** and **10** as well as **14** and **15** were not optimized (1 : 1 molar ratios of **9**/**10** and **14**/**15** were used). It may, however, be assumed that an appropriate molar excess of nucleosides **10** and **15** over difluoromethylene-diphosphonates **9** and **14**, respectively, should result in better yields of the desired dinucleotides **3** and **5**.

In order to distinguish the potential role of the fluorine atom at C-2′ of adenosine versus that in the $-CF_2-$ group on inhibitory activity of the TAD analogues, (**3** and **5**) against dehydrogenases, we also synthesized the pyrophosphate analogue **4**. This compound was prepared, in 50% yield, by the imidazole-catalyzed coupling of tiazofurin 5′-monophosphate[5] with the 2′-deoxy-2′-fluoroadenosine 5′-monophosphate[32].

Although, 2′-deoxy-2′-fluoroadenosine (**15**) was reported more than 20 years ago, the published procedures for its synthesis[30–34] are neither simple nor efficient.



18 R = R′ = H
19 R = Tr, R′ = H
20 R = Tr, R′ = SO_2CF_3

21 R = Ac
22 R = H

23 R = Tr, R′ = THP
15 R = R′ = H

Scheme 3.

For the preparation of **15**, we used the readily accessible 3′-*O*-(tetrahydropyran-2-yl)-N^6-benzoyladenosine[35] (**18**, Scheme 3) as the starting material. Reaction of **18** with chlorotriphenyl methane afforded the 5′-*O*-trityl derivative **19**, which upon treatment with triflyl chloride in dichloromethane followed by sodium acetate in hexamethyl phosphoramide gave the 9-[2-*O*-acetyl-3-*O*-(tetrahydropyran-2-yl)-5-*O*-trityl-β-D-arabinofuranosyl]adenine (**21**). The 2′-*O*-acetyl group of **21** was then selectively removed with 2 M NaOH in pyridine to give **22**. Finally, reaction of **22** with DAST afforded a good yield of the desired 2′-deoxy-2′-fluoroadenosine derivative **23**, which did not contain an *N*-benzoyl protecting group. We had observed similar deacylation during DAST treatment previously[34]. Deprotection of **22** with 80% acetic acid gave **15** in 93% yield.

Mass-spectral analysis.—The structures of dinucleotides **3**, **4**, **5**, and **14** as well as the tetraphosphonate analogues **12** and **17** were confirmed by FABMS. Although, it is known that underivatized nucleosides and nucleotides are best-suited for this analysis, our tetraphosphonates **12** and **17** could not be converted into the free acids because of the instability of the pyrophosphate linkage on Dowex 50 W-X8 (H^+form). Therefore, in order to compare the FABMS spectra of uniformly derivatized compounds, all derivatives were analyzed as the (less convenient) sodium salts.

As expected, the FABMS of dinucleotide pyrophosphate **4** was found to be similar in appearance to that reported earlier for TAD and related analogues[5a,20]. The molecular-weight region of the spectrum establishes the molecular weight of **4** as 471 by the presence of the $[M - H]^-$ ion at m/z 669.9 and associated mono- (m/z 691.8) and di- (713.9) sodium adduct ions. Also evident is the glycerol adduct of the $[M - H]^-$ at m/z 761.8. As in the FAB spectrum of TAD, a number of structurally informative ions[5,20] confirm the identity of **4**. Most significant are the A_1, A_2, B_1, and B_2 fragments representing cleavages of the phosphate bridging-groups (see refs 5b and 20 for the structures of these fragment ions). A series of ions in the m/z 400 to 475 range of the spectrum may be assigned as belonging to the A_2 and B_2 ions series, where the A series contains the thiazole ring and the B series retains the adenine base. Thus, peaks at m/z 409.9, 427.9, 449.9, and 471.9 are identified as $B_2 - H_2O$, B_2, $B_2 + Na$, and $B_2 + 2Na$ ions, respectively. Ions related to the A_2 series appear at m/z 418.9 (A_2) and 440.9 ($A_2 + Na$). No ions of the A_1 or B_1 were observed, possibly because the ion current of the $[M - H]^-$ is split between numerous cationized species.

FABMS confirmed the molecular weight of **3** and **5**. For **3**, ions were observed at m/z 701.9 representing the $[M - H]^-$ ion and sodium-adduct peaks were present at m/z 723.9 ($[M + Na - 2H]^-$) and 745.9 ($[M + 2Na - 3H]^-$). Fragments corresponding to $A_2 + Na$, m/z 481.9 and $B_2 + Na$ were present. As for **5**, the molecular weight is confirmed by the appearance of peaks at m/z 704.0 ($[M - H^-$), 726.1 ($[M + Na - 2H]^-$), and 748.0 ($[M + 2Na - 3H]^-$). Fragments corresponding to $A_2 + Na$ (m/z 474.9) and $B_2 + Na$ (m/z 484.1) are also observed and confirm the substitution of the 2′-hydroxyl group by fluorine in the adenosine portion of

the molecule, as evidenced by a shift of two daltons to a higher mass by the A_2 fragment.

Stabilization of the 5′-phosphate chain limits fragmentation in the FAB spectrum of **14** and only ions assigned to the $[M - H]^-$ (m/z 453.0), $[M + Na - 2H]^-$ (m/z 474.9) and $[M + 2Na - 3H]^-$ (m/z 496.9) species are observed.

Extensive exchange of protons by sodium cations is evident in the molecular-weight region of the FAB spectrum, as indicated by the presence of ions for the following species: $[M - H]^-$ (m/z 902.8), $[M + Na - 2H]^-$ (924.8), $[M + 2Na - 3H]^-$ (m/z 946.8) and $[M + 3Na - 4H]^-$ (m/z 968.8); while the spectrum in this mass range is complex, a molecular weight of 904 for **12** is confirmed. Because of the symmetrical nature of **12** differentiation of fragments arising from one or other ends of the molecule is not possible. However, elimination of one or the other of the adenosine residue leads to an A_2 (or B_2) fragment, observed as multiple sodium-adduct species at m/z 653.8, 675.8, 697.8, and 720.0. Cleavage of the pyrophosphate bond provides an ion at m/z 442.9; however, because of the symmetrical nature of this ion the portion of the molecule carrying the charge cannot be determined.

The FAB mass spectrum of **17** shows a complex pattern in the molecular-weight region due to extensive exchange of protons by sodium ions. Ions representing the adduction of from one to five sodium cations are observed at m/z 910.9, 932.7, 954.6, 976.6 and 998.7. While no $[M - H]^-$ ion is directly observed, the molecular weight of **17** is confirmed by the foregoing ion series. As the thiazole ring-system occupies both the terminal tetraphosphonate positions, A_2 and B_2 designations become the same and ions representing these fragments, as the mono- and di-sodiated species, are observed at m/z 690.7 and 712.8, respectively. Cleavage of the pyrophosphate linkage should produce an ion A_1 or B_1 (in this case $A_1 = B_1$). Unfortunately, sodium cationization shifts the ion to the region of the strong matrix ion at m/z 459, resulting in the masking of this fragment ion. However, the molecule related species and A_2 (B_2) ions serve to establish the identity of compound **17**.

EXPERIMENTAL

General methods.—Melting points were determined on a Thomas–Hoover capillary apparatus and are uncorrected. Column chromatography was performed on Silica Gel G60 (70–230 mesh, ASTM, Merck). TLC was performed on Analtech Uniplates with short-wavelength UV light for visualization. HPLC was performed on a dynamax-60A C18-83-221-C column with a flow rate of 5 mL/min or Dynamax-300A C18-83-243-C column with a flow rate of 20 mL/min of 0.1 M $Et_3N \cdot H_2CO_3$ (TEAB) followed by a linear gradient of 0.1 M TEAB–aq MeCN (70%). Elemental analyses were performed by M – H – W Laboratories, Phoenix, AZ. ^1H NMR spectra were recorded on a Bruker AMX-250 and 400 spectrometer with Me_4Si as the internal standard. Chemical shifts are reported in ppm (δ) and

signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), bs (broad singlet), and dd (double doublet). Values given for coupling constants are first order.

Mass spectrometry.—Negative-ion FABMS were acquired using a double focussing, reverse geometry mass spectrometer (AMD Intectra, Harpsted, Germany) operating at an accelerating voltage of (−) 6 kV. Data acquisition and instrument control were accomplished using two SAM 68K computers (KWS Comoutersysteme GmbH, Ettlingen, Germany). Ionization was effected using a 1.6 mA primary beam of Cs^+ ions accelerated to 7.0 kV above source potential. The mass range 90–2000 daltons was scanned at 60 s/decade at a resolution of 2000. The target temperature was adjusted to 8°C using 1:1 chilled MeOH–H_2O. The samples (5–10 μg) were premixed with 5 μL of glycerol and allowed to stand overnight prior to analysis; one μL of the sample-matrix mixture was applied to the tip of the stainless-steel FAB probe and spectra acquired.

P^1-(Tiazofurin-5′-yl)-P^2-(adenosin-5′-yl)-difluoromethylenediphosphonate (β-difluoromethylene TAD, **3**).—Adenosine 5′-difluoromethylenediphosphonate (340 mg, 0.44 mmol) was stirred with triethyl orthoformate (1 mL) and CF_3CO_2H (1.2 mL) in Me_2SO (5 mL) overnight. The solution was lyophilized to give 272 mg of crude product, which was dissolved in pyridine (10 mL) containing Bu_3N (2.1 mL). To this solution were added 2′,3′-*O*-isopropylidenetiazofurin (**10**, 150 mg, 0.5 mmol) and DCC (400 mg, 2 mmol), and the mixture was stirred for 24 h at 65°C, and concentrated in vacuo. The residue was partitioned between ethyl ether (100 mL) and water (100 mL), the aqueous layer was treated with Dowex 50W-X8 (H^+) for 4 h, the resin was filtered off and the filtrate was lyophilized. The residue was purified by HPLC to give the pyrophosphate derivative **12** as the triethylammonium salt, which was converted into the disodium salt by passing through a column of Dowex 50W-X8 (Na^+ form). The yield of P^1,P^4-di(adenosine 5′-yl)difluoromethylenephosphonate-P^2,P^3-phosphonic acid anhydride (**12**) was 248 mg, 57%. ^{1}H NMR (D_2O) δ 4.40–4.43 (m, 4 H, H-5′, H-5″), 4.51–4.55 (m, 2 H, H-4′), 4.78–4.85 (m, 2 H, H-3′), 4.95 (pseudo-t, 2 H, $J_{1',2'}$ 6.1 Hz, H-2′), 6.0 (d, 2 H, H-1′), 8.08 and 8.17 (two 2 H singlets, H-2, H-8). ^{31}P NMR (D_2O) showed two groups of signals δ −7.0 to −4.3 and 3.0 to 5.4 because of couplings between four mangetically non-equivalent phosphorus atoms and four fluorine atoms.

The desired β-difluoromethylene TAD (**3**) was then eluted from the column and converted into the disodium salt as first described to give 52 mg, 15.1%. ^{1}H NMR (D_2O) δ 4.22–4.44 [m, 8 H, adenosine (A) and tiazofurin (T) H-3′, H-4′, H-5′, H-5″], 4.51 [pseudo-t, $J_{1',2'}$ 4.9, $J_{2',3'}$ 4.0 Hz, 1 H, H-2′(T),] 4.67 [pseudo-t, 1 H $J_{1',2'}$ 5.5, $J_{2',3'}$ 5.2 Hz, H-2′ (A)], 5.10 [d, 1 H, H-1′ (T)], 6.10 [d, 1 H, H-1′(A)], 8.00 (s, 1 H, H-5), 8.20 and 8.48 (two 1 H singlets, H-2, H-8), ^{31}P NMR (D_2O) δ 4.02, 4.36 (AB part of ABX_2 system, $J_{A,B}$ 55.3, $J_{A,X}$ 83.1, $J_{B,X}$ 83.5 Hz, X = F).

2′,3′-O-Isopropylidene-5′-O-tosyltiazofurin (**13**).—To a solution of 2′,3′-*O*-isopropylidenetiazofurin[20] (150 mg, 0.5 mmol) in CH_2Cl_2 (10 mL) containing dimethylaminopyridine (60 mg, 0.5 mmol) and Et_3N (139 μL, 1.0 mmol) was added TsCl

(190 mg, 1 mmol) and the mixture was stirred for 1 h at room temperature. The mixture was concentrated in vacuo and the residue was chromatographed on a silica gel column using $CHCl_3$–EtOH (2%) as the eluent to give **13** (215 mg, 96%) as a foam; ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 3 H, iPr), 1.59 (s, 3 H, iPr), 2.45 (s, 3 H, Ts), 4.17–4.19 (m, 2 H, H-5′, H-5″), 4.40–4.44 (m, 1 H, H-4′), 4.75–4.79 (m, 1 H, H-3′), 4.98–5.02 (m, 1 H, H-2′), 5.20 (d, 1 H, H-1′, $J_{1',2'}$ 3.5 Hz), 5.61 and 7.16 (2 bs, 1 H each, NH_2), 7.32 (d, 2 H, Ts, $J_{H,H}$ 8.6 Hz), 7.72 (d, 2 H, Ts), 8.09 (s, 1 H, H-5). Anal. Calcd for $C_{19}H_{22}N_2O_7S_2$: C, 50.21; H, 4.88; N, 6.16. Found: C, 5.17; H, 4.93; N. 6.07.

(2′,3′-O-Isopropylidenetiazofurin-5′-yl)difluoromethylenediphosphonate (**14**).—Compound **13** (227 mg, 0.5 mmol) and tris(tetra-*n*-butylammonium) hydrogen difluoromethylene-diphosphonate (560 mg, 0.6 mmol) in dry Me_2SO was kept at room temperature for 2 h and then the mixture was lyophilized. The residue was dissolved in water (3 mL) and applied to an HPLC column to give **14** (285 mg, 82%) as the bis(-triethylammonium) salt; ^{1}H NMR (D_2O) δ 1.21 (t, 18 H, CH_3CH_2N), 1.43 (s, 3 H, iPr), 1.63 (s, 3 H, iPr), 3.03 (q, 12 H, CH_3CH_2N), 4.15–4.19 (m, 2 H, H-5′, H-5″), 5.51–5.55 (m, 1 H, H-4′), 5.07 (dd, 1 H, $J_{2',3'}$ 6.3, $J_{3',4'}$ 2.7 Hz, H-3), 5.15 (dd, 1 H, $J_{1',2'}$ 4.1 Hz, H-2), 5.33 (d, 1 H, H-1′), 8.25 (s, 1 H, H-5). ^{31}P NMR (D_2O) δ 3.60, 5.53 (AB part of ABX_2 system, $J_{A,B}$ 54.5, $J_{A,X}$ 79.7, $J_{B,X}$ 85.3 Hz.

Tiazofurin (5′-5″)-2″-deoxy-2″-fluoroadenosine pyrophosphate (**4**).—Tiazofurin 5′-monophosphate[5] (TFMP, 0.12 mmol) was activated with carbonyldiimidazole (100 mg, 0.6 mmol) in DMF (0.4 mL) and then coupled with 2′-deoxy-2′-fluoroadenosine 5′-monophosphate (65 mg, 0.18 mmol) for 3.5 days as described by Marquez and co-workers[5] for coupling of tiazofurin 5′-monophosphate with AMP (method B). Crude **4** was purified first on DEAE Sephadex A-25 (hydrogencarbonate form) with a linear gradient of H_2O–0.1 M TEAB and then by HPLC. Finally, the compound was converted into the sodium salt by passing through a column of Dowex 50W-X8 (Na^+ form). The overall yield of **4** was 50%; ^{1}H NMR (D_2O) δ 4.00–4.40 (m, 8 H, H-2′, H-3′, H-4′, H-5′,5′, H-4″, H-5″,5″), 4.61 (ddd, 1 H, $J_{2'',3''}$ 4.5, $J_{3'',4''}$ 6.8, $J_{3'',F}$ 20.0 Hz, H-3), 4.95 (d, 1 H, $J_{1',2'}$ 4.4 Hz, H-1′), 5.21 (ddd, 1 H, $J_{1'',2''}$ 2.0, $J_{2'',F}$ 52.4 Hz, H-2″), 6.28 (dd, 1 H, $J_{1'',F}$ 16.0 Hz, H-1″), 7.92 (s, 1 H, thiazole), 8.10, 8.28 (two 1 H singlets, H-2, H-8). ^{31}P NMR (D_2O) δ −10.7.

P^1-(Tiazofurin-5′-yl)-P^2-(2′-deoxy-2′-fluoroadenosin-5′-yl)difluoromethylenediphosphonate (**5**).—Compound **14** (100 mg, 0.144 mmol), nucleoside **15** (38 mg, 0.14 mmol), and DCC (297 mg, 1.44 mmol) were dissolved in dry pyridine (3 mL) and kept for 48 h at room temperature. The mixture was concentrated in vacuo, the residue was coevaporated with toluene (2 × 5 mL), dissolved in water (10 mL), and the solution was filtered from dicyclohexylurea. The filtrate was extracted with EtOAc (3 × 5 ml) and lyophilized. The mixture was purified by HPLC to give unreacted **15** (23 mg) and a mixture of two unseparable nucleotides, **16** and isopropylidene protected **17**. The mixture was treated with 10% aq CF_3CO_2H (2.5

mL) for 2 h and lyophilized. Separation on a HPLC column gave the P^1,P^4-di-(tiazofurin-5′-yl)difluoromethylenephosphonate-P^2,P^3-phosphonic acid anhydride (**17**) as the Et_3N salt, which was converted into the tetrasodium salt by passing through a column of Dowex 50-X8 (Na^+ form); yield, 36 mg, 53%; ^1H NMR (D_2O) δ 4.25–4.33 (m, 6 H, H-4′, H-5′,5′), 4.34–4.42 (m, 4 H, H-2′, H-3′), 5.12–5.16 (m, 2 H, H-1′), 8.23 (s, 2 H, H-5). ^{31}P NMR (D_2O) showed two groups of signals δ −7.1 to −4.2 and 2.8 to 5.1. due to couplings between four magnetically nonequivalent phosphorus atoms and four fluorine atoms. The desired dinucleotide **5** was then eluted from the column and converted into the disodium salt to give 5 mg, 4.5%; ^1H NMR (D_2O) δ 4.22–4.55 [m, 8 H, tiazofurin (H-2′, H-3′, H-4′, H-5′,5′), adenosine (H-4′, H-5′,5′)], 4.69 [ddd, 1 H, H-3′ (A), $J_{3',F}$ 20.0, $J_{2',3'}$ 4.2, $J_{3',4'}$ 7.0 Hz, H-3′ (A)], 5.08 [d, 1 H, $J_{1',2'}$ 4.7 Hz, H-1′ (T)], 5.35 [ddd, 1 H, $J_{2',F}$ 54.0, $J_{1',2'}$ 2.0 Hz, H-2′ (A)], 6.38 [dd, 1 H, $J_{1',F}$ 15.8 Hz, H-1′(A)], 8.02 (s, 1 H, H-5), 8.21, 8.42 (two 1 H singlets, H-2, H-8).

3′-O-Tetrahydropyranyl-5′-O-trityl-N^6*-benzoyladenosine* (**19**).—A solution of the less polar isomer of **18**[35] (2.3 g, 5.05 mmol), chlorotriphenylmethane (3.4 g, 12 mmol), and dimethylaminopyridine (600 mg, 5 mmol) in pyridine (100 mL) was stirred for 4 days and then heated at 60°C for 1 h. The mixture was poured into water (300 mL) and extracted with $CHCl_3$ (3 × 150 mL). The organic layer was concentrated, the residue was coevaporated with toluene (3 × 100 mL) and EtOH (2 × 100 mL). The residue was chromatographed on a silica gel column using $CHCl_3$–EtOH (3%) as the eluent to give **19** (3.1 g, 89%); ^1H NMR ($CDCl_3$) δ 1.22–1.87 (m, 6 H, THP), 3.37 (dd, 1 H, $J_{4',5'}$ 4.0, $J_{5',5''}$ 10.5 Hz, H-5′), 3.51–3.57 (m, 2 H, O-CH, THP, H-5″), 4.28–4.30 (m, 2 H, O-CH, THP, OH), 4.31–4.33 (m, 1 H, H-4′), 4.51–4.56 (m, 2 H, O-CH-O, THP, H-3′), 4.80–4.82 (m, 1 H, collapsed to pseudo-t after addition of water, $J_{2',3'}$ 4.8 Hz, H-2′), 6.10 (d, 1 H, $J_{1',2'}$ 4.6 Hz, H-1′), 7.20–7.63 (m, 18 H, Tr, Bz), 8.02–8.05 (m, 2 H, Bz), 8.24, 8.76 (two 1 H singlets, H-2, H-8).

In a similar manner, from the more polar isomer of **18**, the corresponding slower eluting isomer of **19** was obtained; ^1H NMR ($CDCl_3$) δ 1.20–1.79 (m, 6 H, THP), 3.34–3.40 (m, 2 H, O-CH, THP. H-5′), 3.53 (dd, 1 H, $J_{4',5''}$ 3.2, $J_{5',5''}$ 10.5 Hz, H-5″), 3.72–3.75 (m, 1 H, O-CH, THP), 4.46–4.54 (m, 2 H, H-3′, H-4′), 4.73–4.74 (m, 1 H, O-CH-O, THP), 4.82 (pseudo-t, 1 H, $J_{1',2'} = J_{2',3'} = 5.7$ Hz, H-2′), 6.09 (d, 1 H, H-1′), 7.16–7.61 (m, 18 H, Tr, Bz), 8.06 (d, 2 H, J 7.0 Hz, Bz), 8.27, 8.73 (two 1 H singlets, H-2, H-8). Anal. Calcd for $C_{41}H_{39}N_5O_6$: C, 70.57; H, 5.63; N, 10.04. Found: C, 70.42; H, 5.75; N, 10.00.

2′-O-Triflyl-3′-O-tetrahydropyranyl-5′-O-trityl-N^6*-benzoyl-adenosine* (**20**).—To a mixture of the less polar isomer of **19** (4.3 g, 6.17 mmol), dimethylaminopyridine (740 mg, 6.17 mmol), and Et_3N (1.7 mL, 12.34 mmol) in CH_2Cl_2 (100 mL) was added a solution of trifluoromethanesulfonyl chloride (1.3 mL, 12.34 mmol) in CH_2Cl_2 (20 mL). The mixture was stirred for 20 min, and then concentrated in vacuo. The residue was chromatographed on a silica gel column with $CHCl_3$ to give **20** (4.8 g, 94%) as a foam; ^1H NMR ($CDCl_3$) δ 1.45–1.90 (m, 6 H, THP), 3.33

(dd, 1 H, $J_{4',5'}$ 3.6, $J_{5',5''}$ 11.0 Hz, H-5'), 3.45–3.58 (m, 1 H, O-CH, THP), 3.65 (dd, 1 H, $J_{4',5''}$ 3.3 Hz, H-5''), 3.87–3.94 (m, 1 H, O-CH, THP), 4.32 (dt, 1 H, $J_{3',4'}$ 6.3 Hz, H-4'), 4.61–4.63 (m, 1 H, O-CH-O, THP), 5.04 (pseudo-t, 1 H, H-3'), 6.08 (pseudo-t, 1 H, $J_{2',3'}$ 5.1 Hz, H-2'), 6.36 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1'), 7.21–7.49 (m, 15 H, Tr), 7.49–7.67 (m, 3 H, Bz), 8.01 (d, 2 H, *J* 7.0 Hz, Bz) 8.22, 8.74 (two 1 H singlets, H-2, H-8), 9.0 (bs, 1 H, NH).

The slower eluting isomer of **20**; ^{1}H NMR ($CDCl_3$) δ 1.53–1.77 (m, 6 H, THP), 3.32–3.34 (m, 1 H, O-CH, THP), 3.43 (dd, 1 H, $J_{4',5'}$ 4.8, $J_{5',5''}$ 10.9 Hz, H-5'), 3.51–3.53 (m, 1 H, O-CH, THP), 3.64 (dd, 1 H, $J_{4',5''}$ 2.5 Hz, H-5''), 4.45–4.47 (m, 1 H, H-4'), 4.74–4.76 (m, 1 H, O-CH-O, THP), 4.46 (peuso-t, 1 H, $J_{3',4'}$ 5.4 Hz, H-3'), 6.09 (pseudo-t, 1 H, $J_{2',3'}$ 5.4 Hz, H-2'), 6.37 (d, 1 H, $J_{1',2'}$ 3.9 Hz, H-1'), 7.21–7.49 (m, 15 H, Tr), 7.49–7.61 (m, 3 H, Bz), 8.02 (d, 2 H, *J* 7.0 Hz, Bz), 8.22, 8.72 (two 1 H singlets, H-2, H-8), 9.0 (bs, 1 H, NH) Anal. Calcd for $C_{42}H_{38}N_5O_8F_3S$: C, 60.79; H, 4.62; N, 8.44. Found: C, 60.61; H, 4.78; N, 8.40.

9-(2-O-Acetyl-3-O-tetrahydropyranyl-5-O-trityl-β-D-arabinofuranosyl)-N^6-benzoyladenine (**21**).—The faster eluting isomer of **20** (4.15 g, 5 mmol) and NaOAc (3.0 g) in hexamethylphosphoramide (50 mL) were stirred overnight and then partitioned between EtOAc (200 mL) and water (100 mL). The organic layer was washed with water (2 × 100 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed on a column of silica gel with $CHCl_3$–EtOH (3%) as the eluent to give **21** (3.25 g, 88%); ^{1}H NMR ($CDCl_3$) δ 1.50–1.95 (m, 6 H, 3 × CH_2, THP), 1.72 (s, 3 H, Ac), 3.40–3.60 (m, 3 H, O-CH, THP, H-5', H-5''), 3.80–3.92 (m, 1 H, O-CH, THP), 4.23 (q, 1 H, $J_{4',5'} = J_{4',5''} = 4.5$ Hz, H-4'), 4.53 (dd, 1 H, $J_{3',4'}$ 4.4 Hz, H-3'), 4.71 (t, 1 H, *J* 3.2 Hz, O-CH-O, THP), 5.56 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-2'), 6.65 (d, 1 H, $J_{1',2'}$ 4.6 Hz, H-1'), 7.20–7.62 (m, 18 H, Bz, Tr), 8.03 (d, 2 H, *J* 7.0 Hz, Bz), 8.16, 8.77 (two 1 H singlets, H-2, H-8).

The more-polar isomer of **21**; ^{1}H NMR ($CDCl_3$) δ 1.50–1.75 (m, 6-H, 3 × CH_2, THP), 1.77 (s, 3 H, Ac), 3.40–3.46 (m, 3 H, O-CH, THP, H-5',5''), 3.58–3.61 (m, 1 H, O-CH, THP), 4.33 (q, 1 H, $J_{4',5'} = J_{4',5''} = 4.8$ Hz, H-4'), 4.56 (dd, 1 H $J_{3',4'}$ 4.0 Hz, H-3'), 4.86–4.89 (m, 1 H, O-CH-O, THP), 5.33 (dd, 1 H, $J_{2',3'}$ 1.8 Hz, H-2'), 6.65 (d, 1 H, $J_{1',2'}$ 4.2 Hz, H-1') 7.23–7.62 (m, 18 H, Bz, Tr), 8.03 (d, 2 H, *J* 7.0 Hz, Bz), 8.21 8.78 (two 1 H singlets, H-2, H-8). Anal. Calcd for $C_{43}H_{41}N_5O_7$: C, 69.81; H, 5.59; N, 9.47. Found: C, 69.77; H, 5.66; N, 9.41.

9-(3-O-Tetrahydropyranyl-5-O-trityl-β-D-arabinofuranosyl)-N^6-benzoyladenine (**22**).—Compound **21** (faster eluting isomer, 739 mg, 1 mmol) was dissolved in 2:1 EtOH-pyridine (4 mL), cooled in ice. To this mixture was added 2 M aq NaOH (1.25 mL), and the reaction was monitored by TLC ($CHCl_3$–EtOH, 2%) and quenched by addition of Dowex 50 (H^+ form) when the reaction was complete (15–25 min). The resin was filtered and washed with a mixture of EtOH–pyridine, and the filtrate and washings were concentrated in vacuo. The residue was chromatographed on a silica gel column with $CHCl_3$–EtOH (1%) to give **22** (613 mg, 88%); ^{1}H NMR ($CDCl_3$) δ 1.47–1.80 (m, 6 H, THP), 3.35–3.47 (m, 2 H, O-CH, THP, H-5'), 3.55–3.72 (m, 2 H, O-CH, THP, H-5''), 4.21–4.25 (m, 1 H,

H-4′), 4.30–4.42 (m, 2 H, O-CH-O, THP, H-3′), 4.78 (pseudo-t, $J_{1',2'} = J_{2',3'} = 2.0$ Hz, H-2′), 6.40 (d, 1 H, H-1′), 7.30–7.70 (m, 18 H, Tr, Bz), 8.03 (d, 2 H, J 7.2 Hz, Bz), 8.31, 8.72 (two 1 H singlets, H-2, H-8).

The slower-eluting isomer of **22**; ^{1}H NMR ($CDCl_3$) δ 1.50–1.79 (m, 6 H, $3 \times CH_2$, THP), 3.38–3.41 (m, 2 H, $J_{4',5'}$ 3.8, $J_{5',5''}$ 10.6 Hz, O-CH, THP, H-5′), 3.59–3.64 (m, 1 H, O-CH, THP), 3.70 (dd, 1 H, $J_{4',5''}$ 2.9 Hz, H-5″), 4.26 (q, 1 H, $J_{4',5'} = J_{4',5''} = 3.1$ Hz, H-4′), 4.33–4.39 (m, 2 H, O-CH-O, THP, H-3′), 4.74 (pseudo-t, 1 H, $J_{2',3'}$ 1.8 Hz, H-2′), 6.37 (d, 1 H, $J_{1',2'}$ 3.0 Hz, H-1′), 7.23–7.62 (m, 18 H, Bz, Tr), 8.03 (d, 2 H, J 7.2 Hz, Bz), 8.47, 8.75 (two 1 H singlets, H-2, H-8). Anal. Calcd for $C_{41}H_{39}N_5O_6$: C, 70,57; H, 5.63; N, 10.04. Found: C, 70.52; H, 5.59; N, 10.12.

2′-Deoxy-2′-fluoro-3′-O-tetrahydropyranyl-5′-O-trityl-adenosine (**23**).—To a solution of DAST (635 μL, 6 equiv) in CH_2Cl_2 (5 mL) containing pyridine (500 μL) was added a solution of **22** (560 mg, 0.8 mmol, slower eluting isomer) in CH_2Cl_2 (5 mL). The mixture was stirred at room temperature overnight, and then diluted with CH_2Cl_2 (100 mL). The solution was washed with 5% $NAHCO_3$ (20 mL) and water (20 mL), dried, and concentrated in vacuo. The residue was chromatographed on a silica gel column with $CHCl_3$–EtOH (2%) to give **23** (350 mg, 58.8%) as a foam; ^{1}H NMR ($CDCl_3$) δ 1.39–1.73 (m, 6 H, THP), 3.37–3.43 (m, 2 H, O-CH, THP, H-5′), 3.52–3.58 (m, 1 H, O-CH, THP), 3.64 (dd, 1 H, $J_{4',5'}$ 2.8, $J_{5',5''}$ 10.8 Hz, H-5″), 4.40–4.42 (m, 1 H, H-4′), 4.79 (dt, 1 H, $J_{3',4'}$ 3.5, $J_{3',F}$ 12.3 Hz, H-3′). 4.80–4.81 (m, O-CH-O, THP), 5.60 (dt, 1 H, $J_{2',3'}$ 4.3, $J_{2',F}$ 53.8 Hz, H-2′), 6.17 (bs, 2 H, NH_2), 6.32 (dd, 1 H, $J_{1',2'}$ 2.7, $J_{1',F}$ 15.6 Hz, H-1′), 7.22–7.49 (m, 15 H, Tr), 8.15, 8.32 (two 1 H singlets, H-2, H-8).

In a similar manner, a faster-eluting isomer of **23** was treated with DAST to give a crude **23**, which without purification was further converted into **15**. Anal. Calcd for $C_{34}H_{34}FN_5O_4$: C, 68.55; H, 5.75; N, 11.75. Found: C, 68.60; H, 5.77; N, 11.81.

2′-Deoxy-2′-fluoroadenosine (**15**).—Compound **23** (595 mg, 1 mmol) was dissolved in 80% AcOH and kept overnight. The mixture was concentrated in vacuo and the residue was crystallized from EtOH to give **15** (250 mg, 73%); mp 230–232°C (lit.[32]). The ^{1}H NMR spectrum was identical with that of an authentic sample.

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