SYNTHESES OF [2-²H]-5-ETHYNYL-1-(β-D-RIBOFURANOSYL)

IMIDAZOLE-4-CARBOXAMIDE AND 5-ETHYNYL-1-([5-³H]-β-D-

RIBOFURANOSYL)IMIDAZOLE-4-CARBOXAMIDE (EICAR)

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SUMMARY

Metallation of 5-ethynyl-1-(2,3,5-tri-*O-tert*-butyldimethylsilyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**1**) using n-BuLi, deuteration with deuterium oxide and removal of the *tert*-butyldimethylsilyl protecting groups using tetrabutylammonium fluroide yielded [2-²H]-5-ethynyl-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (**5a**, 75 atom % deuterium). Regiospecific deprotection of the *masked aldehyde N,N'-diphenylethylenediamino synthon* **14** using DIAION PK212 ion-exchange resin (H⁺ form) yielded the aldehyde derivative (**15**). Reduction of the aldehyde moiety of **15** using excess [³H]NaBH₄ gave the carbinol product **17**. Removal of the ribofuranosyl 2,3-isopropylidene protecting group from **17** using 90% trifluoroacetic acid afforded 5-ethynyl-1-([5-³H]-β-D-ribofuranosyl)imidazole-4-carboxamide (**18**, 19% chemical yield, > 99% radiochemical purity, specific activity 1.56 Ci/mmol).

Key words: Deuteration, [2-²H]-5-ethynyl-1-(β-D-ribofuranosyl)imidazole-4-carboxamide, [³H]sodium borohydride reduction, 5-ethynyl-1-([5-³H]-β-D-ribofuranosyl)-imidazole-4-carboxamide, EICAR.

INTRODUCTION

EICAR [5-ethynyl-1-(β-D-ribofuranosyl)imidazole-4-carboxamide, **3**] has been shown to

exhibit *in vitro* cytostatic activity against various tumor cell lines including human solid

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tumor cells and *in vivo* antitumor activity against murine leukemias such as L1210 and P388 (1-4). In addition to its cytostatic activity, EICAR also exhibits an antiviral activity spectrum that is similar to ribavirin (5-7). Inhibition of inosine 5'-monophosphate (IMP) dehydrogenase, which catalyzes the oxidative conversion of IMP to xanthosine 5-monophosphate (XMP) and is one of the most prominent rate-controlling enzymes of *de novo* guanine biosynthesis in mammalian systems, is believed to be responsible for the cytostatic and antiviral activities observed (8,9). Recent studies indicated that EICAR 5'-monophosphate irreversibly inactivated both human type II and *E. coli* IMP dehydrogenase (10). In the course of ongoing metabolic and mechanistic studies, it became necessary to prepare [^{2}H]- and [^{3}H]-labelled EICAR. We now report facile procedures for the syntheses of [2-^{2}H]-5-ethynyl-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (**5a**) and high specific activity 5-ethynyl-1-([5-^{3}H]-β-D-ribofuranosyl)imidazole-4-carboxamide (**18**).

RESULTS AND DISCUSSION

In selecting a synthetic procedure for the synthesis of [^{2}H]-labelled EICAR, it was expected that metallation of 5-ethynyl-1-(2,3,5-tri-*O-tert*-butyldimethylsilyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**1**) at the terminal position of the 5-ethynyl moiety using lithium diisopropylamide (LDA), deuteration with CD$_3$OD and removal of the TBDMS protecting groups using tetra-n-butylammonium fluoride (TBAF) would yield the target, 5-[2-^{2}H]-ethynyl-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (see Scheme 1). Accordingly, reaction of **1** with two equivalents of LDA at -70 °C, followed by quenching with CD$_3$OD, yielded the 5-[2-^{2}H]-ethynyl product **2** in 81% chemical yield as a colorless oil. A comparison of the integrals for the imidazole C-2H at δ 7.92 and the acetylene C≡C*H* at δ 3.76 in the ^{1}H nmr spectrum of **2** indicated that the extent of deuterium incorporation on the ethynyl moiety (C≡C*D*) was about 85%. However, treatment of **2** with TBAF, to remove the TBDMS protecting groups, resulted in a complete exchange of the labile deuterium atom by a proton to afford unlabeled EICAR (**3**).

An alternative approach to incorporate either a deuterium or a tritium substituent at the C-2 position of the imidazole ring, which would be chemically and metabolically stable, was investigated (see Scheme 1). Thus, treatment of **1** with five equivalents of n-BuLi in THF at -70 °C and quenching the reaction with D_2O yielded the *bis*-deuterated product **4a** in 75% chemical yield as a colorless oil. Comparison of the integrals for the imidazole C-2H and the ethynyl proton ($C{\equiv}CH$) in the ^1H nmr spectrum of **4a**, with the correspondiong protons in the ^1H nmr spectrum of **1**, indicated that the extent of deuterium incorporatation at the imidazole C-2 position and on the 5-ethynyl substituent were 75% and 50%, respectively. Desilylation of **4a** using TBAF afforded $[2{-}^2H]{-}5{-}ethynyl{-}1{-}(\beta{-}D{-}ribofuranosyl)imidazole{-}4{-}carboxamide$ (**5a**) as a white solid in 56% chemical yield. The extent of deuterium incorporation at the C-2

Scheme 1

4a, R = ^2H (deuterium)
4b, R = ^3H (tritium)

5a, R = ^2H (deuterium)
5b, R = ^3H (tritium)
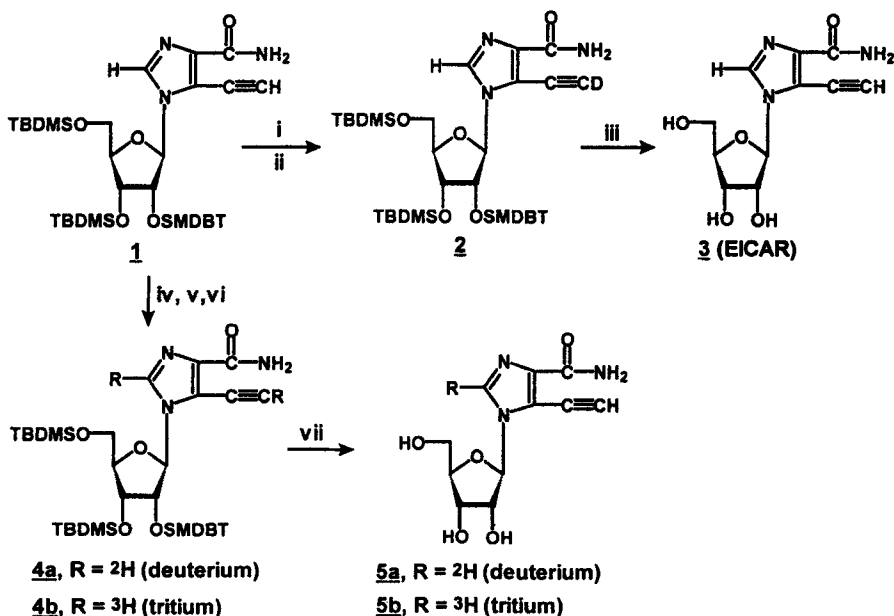
Reagents: i, LDA, THF, -70 °C; ii, CD_3OD, -70 °C; iii, TBAF, THF, 25 °C, 30 min; iv, n-BuLi, THF, -70 °C, 1 h; v, $[^2H]H_2O$ (**4a**) or $[^3H]H_2O$ (**4b**), -70 °C, 1h; vi, AcOH (-70 → 25 °C); vii, TBAF, 25 °C, 30 min.

position of **5a** was estimated by ^{1}H nmr spectrometry to be 75% which indicates that the C-2D subsituent was stable during the desilylation reaction using TBAF. It was expected that a similar reaction using [^{3}H]H$_2$O, in the place of [^{2}H]H$_2$O, would yield [2-^{3}H]EICAR (**5b**) having a theroretical specific activity of at least 70 mCi/mmol. However, reaction of **1** (21.7 mg, 0.0356 mmol) with 6.4 molar equivalents of *n*-BuLi (0.229 mmol), quenching with [^{3}H]H$_2$O (100 μL, specific activity 28 Ci/mL) and deblocking using TBAF afforded [2-^{3}H]EICAR (**5b**, 39% chemical yield, 12.4 μCi total radioactivity, specific activity 0.87 mCi/mmol, > 99% radiochemical purity). The low radiochemical yield and low specific activity of 2-[^{3}H]EICAR is most likely due to a significant isotope effect resulting from competition between the reaction of the imidazole C-2Li species with [^{1}H]H$_2$O relative to [^{3}H]H$_2$O. This explanation is in agreement with the fact that larger mass isotopes generally react slower than lower mass isotopes. Consequently, this synthetic method is not suitable for the synthesis of [2-^{3}H]EICAR (**5b**) since very high specific activity [^{3}H]H$_2$O is not commercially available.

An alternative synthetic strategy (see Scheme 3) was therefore investigated to circumvent the limitation encountered in the reaction using [^{3}H]H$_2$O for the synthesis of [2-^{3}H]EICAR **5b** (as illustrated in Scheme 1). The C-5 position of the β-D-ribofuranosyl ring system (**18**) was selected for incorporation of the tritium label since this is expected to be a metabolically stable position (see Scheme 3). It was anticipated that the target [^{3}H]-labelled EICAR (**18**) could be prepared readily from the aldehyde **15** by reduction with high specific activity [^{3}H]NaBH$_4$. Although the aldehyde **15** should be accessible by oxidation of the hydroxymethyl substituent present in **16**, it was expected that simultaneous dehydration of the 4-carbamoyl moiety to a 4-cyano substituent would also occur. Therefore, the 4-cyano derivative **11** was selected as the starting material, which was readily prepared from the known 4-carboxamido-5-amino derivative **6** (11). Compound **6** was elaborated to the required starting reagent **11** using previously reported methods (1,2), as illustrated in Scheme

2. Moffatt oxidation (12) of the hydroxymethyl moiety of **11** using 1,3-dicyclohexylcarbodiimide in DMSO (DCC/DMSO) gave aldehyde **12**, which was presumed to be unstable, and so was converted to the *masked* aldehyde *N,N'*-diphenylethylenediamino derivative **13** in 67% yield from **11**. Hydrolysis of the 4-cyano group of **13** afforded thecorresponding carboxamide derivative **14** in 78% yield. Removal of the *N,N'*-diphenylethylenediamino protecting group present in **14** using a cation exchange resin (H⁺ form) in aqueous THF yielded the aldehyde **15**, which without further purification, was reduced using NaBH₄ to afford 5-ethynyl-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide (**16**) in 59% overall yield. Removal of the 2',3'-*O*-isopropylidene protecting group of **16** by treatment with 90% trifluoroacetic acid (TFA) yielded unlabeled EICAR (**3**).

Scheme 2
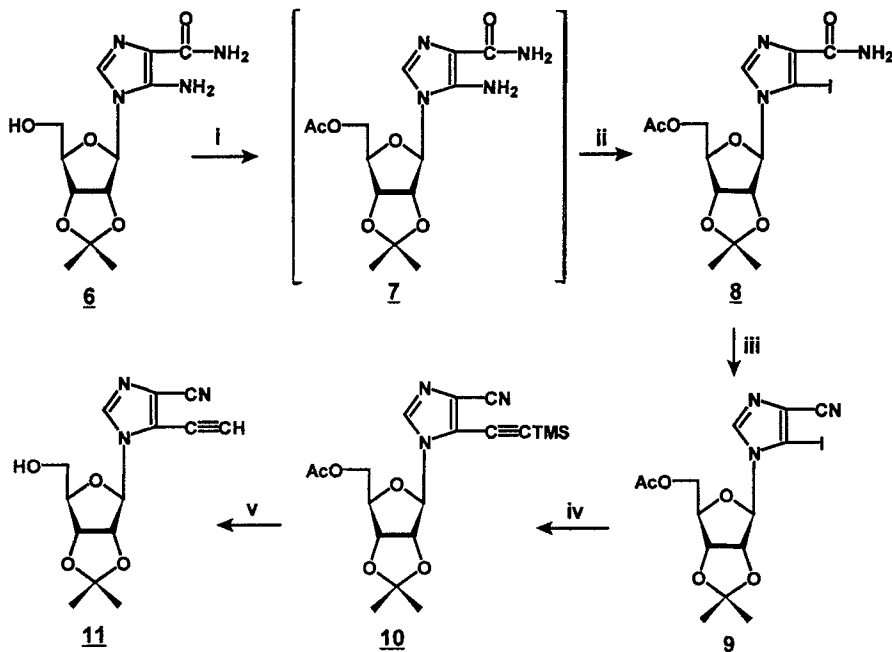
Reagents: i, Ac₂O, DMAP, Et₃N, MeCN, 25 °C, 20 min; ii, CH₂I₂, isoamyl nitrite, 100 °C, 20 min; iii, POCl₃, Et₃N, CH₂Cl₂, 0 °C, 1 h; iv, bis(benzonitrile)palladium dichloride, TMS-C≡C-Sn-n-Bu₃, MeCN, 100 °C, 10 h; v, NH₃-MeOH, 25 °C, 2 h.

The methodology developed for the synthesis of unlabeled EICAR (3) (see Scheme 3) was used for the synthesis of [³H-]-labeled EICAR (18). Thus, regiospecific deprotection of the *masked aldehyde synthon* (14) using DIAION PK212 ion-exchange resin (H⁺ form) suspended in aqueous THF at 25 °C and a reaction time of 30 minutes yielded the aldehyde

Scheme 3
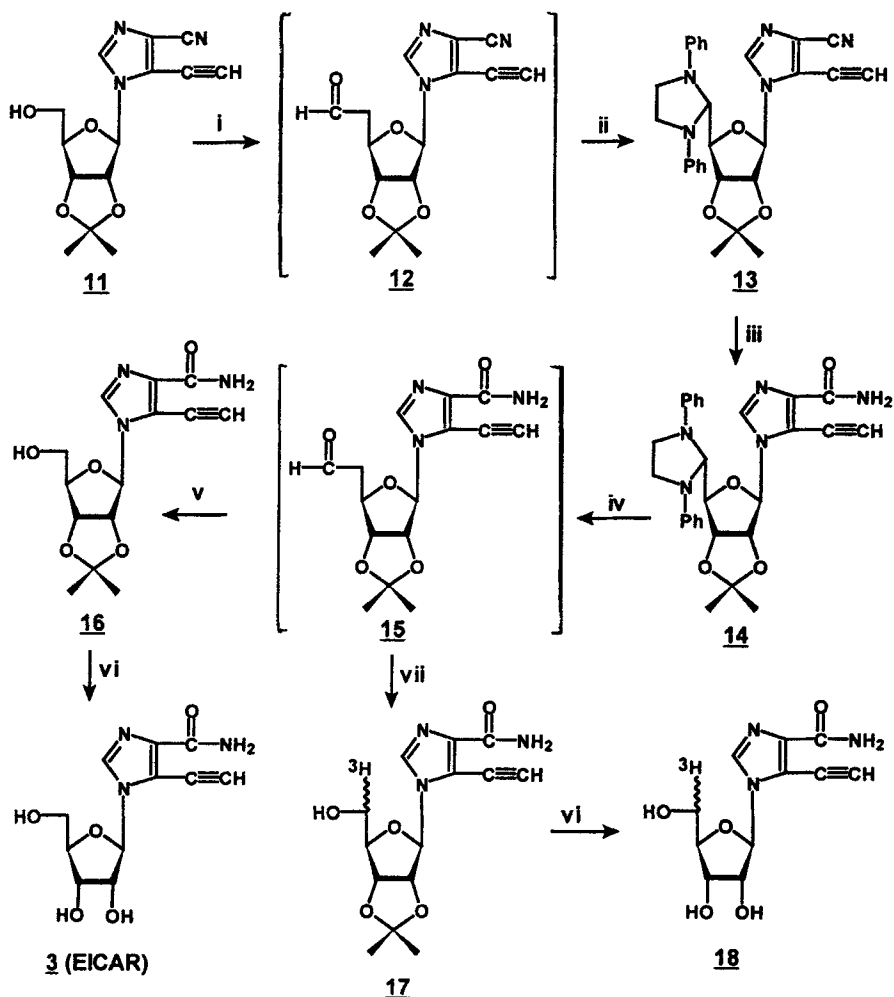
Reagents: i, DCC, DMSO, Cl₂CHCO₂H, oxalic acid; ii, *N,N'*-diphenylethylenediamine, 25 °C, 40 min; iii, NH₄OH-MeOH, H₂O₂, 25 °C, 7 h; iv, DIAION PK212 (H⁺ form), THF, H₂O, 25 °C, 30 min; v, NaBH₄, MeOH, 25 °C, 20 min; vi, 90% CF₃CO₂H, 25 °C, 10 min; vii, [³H]NaBH₄, MeOH, 25 °C, 30 min.

derivative (**15**). Reduction of **15** using excess [³H]NaBH₄ (0.5 molar equivalents, specific

activity 8.3 Ci/mmol) gave the tritiated carbinol (**17**) which was then treated with 90% TFA

to remove the 2',3'-*O*-isopropylidene protecting group to afford 5-ethynyl-1-([5-³H]-β-D-

ribofuranosyl)imidazole-4-carboxamide (**18**, 19% chemical yield, 7 mCi total radioactivity,

specific activity 1.56 Ci/mmol, > 99% radiochemical purity). A two-fold excess of

[³H]NaBH₄ (assuming four tritide anions are available for reduction of the aldehyde group)

was utilized to simplify the radiosynthesis procedure. This allowed one to weigh more

accurately a larger quantity of [³H]NaBH₄, use a smaller quantity of the *masked aldehyde*

synthon (**14**), and to ensure that the reduction reaction proceeded in high yield. Despite a

modest 19% chemical yield of the target product **18**, the high specific activity achieved (1.56

Ci/mmol) approached the theoretical maximum of 2 Ci/mmol.

SUMMARY

[2-²H]-5-Ethynyl-1-(β-D-ribofuranosyl)imidazole-4-carboxamide {[2-²H]EICAR, **5a**, 75

atom % deuterium} was synthesized in 42% overall chemical yield from 5-ethynyl-1-(2,3,5-

tri-*O*-tert-butyldimethylsilyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**1**) using a two

step reaction sequence. High specific activity (1.56 Ci/mmol) 5-ethynyl-1-([5-³H]-β-D-

ribofuranosyl)imidazole-4-carboxamide (EICAR, **18**) was synthesized in 14% radiochemical

yield from the *masked aldehyde synthon* **14** using a facile three step reaction sequence.

EXPERIMENTAL

All reagents used were analytical grade. Anhydrous tetrahydrofuran (THF) was dried by

distillation from calcium hydride just prior to use. Qualitative thin layer chromatography

(TLC) was performed using Whatman MK6F microslides (0.25 mm thickness). Preparative

TLC separations were effected using Whatman TLC plates (20 x 20 cm, 1 mm thickness).

High pressure liquid chromatography (HPLC) was performed using a Waters HPLC system

comprised of two Model 501 solvent pumps, Model 860 gradient flow controller, Model

U6K injector and Model 486 variable wavelength ultraviolet detector set at 263 nm using a

Waters μBondapak C18 column (3.9 mm x 300 mm, P/N 27324) with MeOH-H$_2$O (1:1, v/v)

as eluent at a flow rate of 0.5 mL/min. The identity of [^{3}H]-labelled EICAR (**5b**, **18**) was

confirmed by comparison of their reverse phase HPLC retention time with an unlabeled

authentic reference standard. Radioactivity was determined by liquid scintillation counting

using 10 mL OptiPhase 'HiSafe' fluor with a Beckman LS9000 liquid scintillation counter.

Ultraviolet spectra (UV) and quantitative UV determinations were performed using a Philips

Model 8740 UV spectrophotometer in EtOH-H$_2$O (1:49, v/v). [^{3}H]H$_2$O (specific activity 28

Ci/mL) and [^{3}H]NaBH$_4$ (specific activity 8.3 Ci/mmol) were obtained from Amersham. 5-

Ethynyl-1-(2,3,5-tri-*O-tert*-butyldimethylsilyl-β-D-ribofuranosyl)imidazole-4-carboxamide

(**1**) was prepared according to the literature procedure (13).

[2-^{2}H]-5-Ethynyl-1-(2,3,5-tri-*O-tert*-butyldimethylsilyl-β-D-ribofuranosyl)imidazole-4-

carboxamide (4a).

A solution of **1** (13) (305 mg, 0.5 mmol) in dry THF (15 mL) was placed in a two-necked

flask equipped with a gas inlet adaptor, thermometer, and rubber septum. To this, a solution

of n-BuLi in hexane (1.5 mL of 1.62 M, 2.5 mmol) was added at a rate such that the reaction

temperature did not exceed -70 °C. After the mixture was stirred for 1 hour at below -70 °C,

D$_2$O (1 mL) was added and the whole was stirred for a further 1 hour. The reaction was then

quenched by adding AcOH (1 ml) and the temperature was allowed to rise to 25 °C. The

reaction mixture was concentrated *in vacuo* and the residue was dissolved in EtOAc (50 mL).

The solution was washed successively with H$_2$O (15 mL), saturated aqueous NaHCO$_3$ (15

mL) and saturated brine (15 mL). The separated organic layer was dried (Na$_2$SO$_4$) and

concentrated to dryness *in vacuo*. The residue was purified on a silica gel column (2.6 x 11

cm), eluted with 25-50% EtOAc in hexane, to give deuterated **4a** (230 mg, 75% as a

colorless oil).

Integration of the ^{1}H nmr spectrum of **4a** indicated that the extent of deuterium incorporation at the C-2 position of the imidazole ring and on the 5-ethynyl moiety was about 75 and 50 atom % deuterium, respectively.

[2-^{2}H]-5-Ethynyl-1-(β-D-ribofuranosyl)imidazole-4-carboxamide {[2-^{2}H]EICAR, 5a}.

A THF solution of TBAF (1.44 mL of 1 M, 1.44 mmol) was added to a solution of **4a** (220 mg, 0.36 mmol) in THF (5 mL). The mixture was stirred for 30 min at 25 °C, and concentrated *in vacuo*. The residue was dissolved in H_2O (20 mL), which was washed with ether (3 x 20 mL). The aqueous layer was concentrated to dryness *in vacuo* and the residue was purified on a silica gel column (2.6 x 10 cm), eluted with 5-30% EtOH in CHCl$_3$, to give **5a** (54 mg, 56% as a white solid).

Integration of the ^{1}H nmr spectrum of **5a** indicated that there was about 75 atom % deuterium at the C-2 position of the imidazole ring.

[2-^{3}H]-5-Ethynyl-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (5b).

n-Butyllithium (0.143 mL of a 1.6 M solution in hexane, 0.229 mmol, 6.4 equivalents) was added to a solution of **1** (21.7 mg, 0.0356 mmol) in dry THF (1 mL) precooled to -65 °C under an argon atmosphere with stirring. The reaction mixture was maintained at -65 °C for 1 hour, [^{3}H]H_2O (0.1 mL, specific acitivity 28 Ci/mL) was added, the reaction mixture was stirred for 1 hour at -65 °C, AcOH (50 µL) was added to quench the reaction and the stirred reaction mixture was allowed to warm to 25 °C. This solution was applied onto a Waters C-18 sep-pak cartridge column that was eluted with H_2O (3 mL) and then EtOAc (6 mL). A small aliquot (about 0.1 µL) of each fraction collected (0.4 mL) was applied to a Whatman MK6F silica gel microslide that was not developed. The solvent, from those fractions which exhibited a UV visible spot (254 nm) on the silica gel microslide above, was removed *in vacuo* to afford **4b** as a solid which was used immediately in the subsequent reaction without further purification. A solution of **4b**, obtained above, in THF (0.5 mL) and a solution of TBAF (1M in THF, 142 µL) was stirred for 30 minutes at 25 °C, the solvent was removed *in vacuo* and the residue obtained was dissolved in H_2O (0.5 mL). An aqueous solution of 1 M

NaClO$_4$ (152 µL) was added and the resulting precipitate was removed by filtration. The filtrate obtained was washed with ether (2 x 3 mL), the ether wash was discarded, and the volume of the aqueous fraction was reduced until a precipitate formed that was removed by filtration and discarded. The aqueous filtrate was then applied onto a Waters C-18 sep-pak cartridge column which was eluted with H$_2$O (15 mL). A small aliquot (about 0.1 µL) of each fraction collected (0.4 mL) was applied to a Whatman MK6F silica gel microslide that was not developed. The solvent, from those fractions which exhibited a UV visible spot (254 nm) on the silica gel microslide above, was removed *in vacuo* to afford **5b** which was formulated as a solution in EtOH-H$_2$O (1:49, v/v, 1.4 mL). The radiochemical purity of this solution of **5b** was determined by HPLC analysis and liquid scintillation counting (LSC). All of the radioactivity was associated with the eluting peak collected at 5.9 minutes (12.4 µCi total radioactivity, > 99% radiochemical purity), which was identical to the retention time of an authentic sample of unlabeled EICAR (**3**). The UV spectrum exhibited by the solution of [³H]-**5b** was also identical to that for an unlabeled authentic sample of **3**. Quantitative UV analysis of the solution of [³H]-**5b** described above indicated that the chemical yield was 39% (3.7 mg) and that the specific activity was 0.87 mCi/mmol.

5-Iodo-1-(5-*O*-acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide (8).

Triethylamine (8.3 mL, 60 mmol) was added to a solution of **6** (11) (11.92 g, 40 mmol) in dry acetonitrile (100 mL) containing acetic anhydride (5.6 mL, 60 mmol) and DMAP (50 mg). The reaction mixture was stirred for 20 minutes at 25 °C and EtOH (10 mL) was added to the mixture to decompose the excess acetic anhydride. The mixture was concentrated to dryness *in vacuo* and the residue was dissolved in CHCl$_3$ (400 mL), which was washed with saturated aqueous NaHCO$_3$ (2 x 100 mL), followed by saturated brine (100 ml). The separated organic layer was dried (Na$_2$SO$_4$) and concentrated to dryness to give crude **7**. A CH$_2$Cl$_2$ solution of **7** (40 mL) was added dropwise to a solution of diiodomethane (150 mL) containing isoamyl nitrite (15 mL, 112 mmol) at 100 °C. After being stirred for 20 minutes,

the reaction mixture was absorbed onto a silica gel column (5.8 x 27 cm), which was washed

with CHCl$_3$ to remove diiodomethane, and then eluted with 1-4 % EtOH in CHCl$_3$, to give **8**

(10.39 g, 58% as a yellow foam): MS *m/z* 451 (M$^+$); ^{1}H nmr (CDCl$_3$) δ 7.86 (s, 1H, H-2),

7.04 (br s, 1H, amide proton), 5.98 (d, 1H, H-1', $J_{1',2'}$ = 2.8 Hz), 5.48 (br s, 1H, amide proton),

4.86 (dd, 1H, H-2', $J_{1',2'}$ = 2.8, $J_{2',3'}$ = 6.3 Hz), 4.79 (dd, 1H, H-3', $J_{2',3'}$ = 6.3, $J_{3',4'}$ = 3.8 Hz),

4.47 (m, 1H, H-4'), 4.34 (m, 2H, H-5'a, b), 2.10 (s, 3H, COCH_3), 1.38 and 1.64 (two s, 3H

each, C*Me*$_2$).

5-Iodo-1-(5-*O*-acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carbonitrile

(9).

Phosphorus oxychloride (0.56 mL, 6.0 mmol) was added dropwise to a solution of **8** (1.8 g,

4.0 mmol) in dry CH$_2$Cl$_2$ (30 mL) containing Et$_3$N (2.79 mL, 20 mmol) at 0 °C and the whole

was stirred for 1 hour at 0 °C. The reaction was quenched by addition of crushed ice. The

solution was diluted with CHCl$_3$ (80 mL), which was washed successively with H$_2$O (2 x 40

ml), saturated aqueous NaHCO$_3$ (40 mL) and saturated brine (40 mL). The separated organic

layer was dried (Na$_2$SO$_4$) and concentrated to dryness *in vacuo*. The residue was purified on a

silica gel column (3.6 x 9 cm), eluted with 25-50% EtOAc in hexane, to give **9** (1.52 g, 88%

as a yellow oil): MS *m/z* 433 (M$^+$); ^{1}H nmr (CDCl$_3$) δ 7.86 (s, 1H, H-2), 5.83 (d, 1H, H-1',

$J_{1',2'}$ = 2.0 Hz), 4.80 (m, 2H, H-2', H-3'), 4.52 (m, 1H, H-4'), 4.34 (m, 2H, H-5'a,b), 2.08 (s,

3H, COCH_3), 1.34 and 1.69 (two s, 3H each, C*Me*$_2$).

5-Trimethylsilylethynyl-1-(5-*O*-acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)

imidazole-4-carbonitrile (10).

A mixture of **9** (433 mg, 1.0 mmol), *bis*(benzonitrile)palladium dichloride (36 mg, 10 mol%),

trimethyl[(tributylstannyl)ethynyl]silane (580 mg, 1.5 mmol) in dry acetonitrile (5 mL) in a

sealed glass tube was heated at 100 °C for 10 hours. The reaction mixture was filtered

through a Celite pad and washed with EtOH. The combined filtrate and washings were

concentrated to dryness *in vacuo* and the residue was purified on a silica gel column (2.8 x 12

cm), eluted with 25-33% EtOAc in hexane, to give **10** (349 mg, 87% as a brown oil): MS *m/z*

403 (M$^+$); ^{1}H nmr (CDCl$_3$) δ 7.66 (s, 1H, H-2), 5.91 (d, 1H, H-1', $J_{1',2'}$ = 2.4 Hz), 4.95 (dd,

1H, H-2', $J_{1',2'}$ = 2.4, $J_{2',3'}$ = 6.3 Hz), 4.75 (dd, 1H, H-3', $J_{2',3'}$ = 6.3, $J_{3',4'}$ = 3.2 Hz), 4.55 (m, 1H,

H-4'), 4.30 (m, 2H, H-5'a, b), 2.01 (s, 3H, COC*H*$_3$), 1.37 and 1.60 (two s, 3H each, C*Me*$_2$),

0.30 (s, 9H, Si*Me*$_3$).

5-Ethynyl-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carbonitrile (<u>11</u>).

Compound <u>10</u> (343 mg, 0.85 mmol) was dissolved in NH$_3$/MeOH (saturated at 0 °C, 10 mL),

and the mixture was stirred for 2 hours at 25 °C. The solvent was removed *in vacuo* and the

residue was purified on a silica gel column (2.6 x 8 cm), eluted with 50-75% EtOAc in

hexane, to give <u>11</u> (242 mg, 98% as a brown oil): MS *m/z* 289 (M$^+$); ^{1}H nmr (CDCl$_3$) δ 8.06

(s, 1H, H-2), 5.93 (d, 1H, H-1', $J_{1',2'}$ = 3.3 Hz). 4.94 (m, 2H, H-2', H-3'), 4.46 (br s, 1H, H-4'),

3.91 (m, 2H, H-5'a, b), 3.89 (s, 1H, C≡C*H*), 3.46 (dd, 1H, 5'-OH, *J* = 3.8, 4.4 Hz), 1.38 and

1.60 (two s, 3H each, C*Me*$_2$).

5-Ethynyl-1-[5-deoxy-5,5-(*N,N'*-diphenylethylenediamino)-2,3-*O*-isopropylidene-β-D-ribofuranosyl]imidazole-4-carbonitrile (<u>13</u>).

Compound <u>11</u> (289 mg, 1.0 mmol) and DCC (618 mg, 3.0 mmol) were dissolved in dry

DMSO (5 mL) and the mixture was cooled in an ice bath under an argon atomosphere.

Dichloroacetic acid (41 μL, 0.5 mmol) was added to the mixture and the whole was stirred

for 1.5 hours at 25 °C. To the mixture, a MeOH solution (2 mL) of oxalic acid (180 mg, 2.0

mmol) was added dropwise and stirred for 30 minutes. The reaction mixture was filtered

through a Celite pad and the residue was washed with ice-cold MeOH. To the combined

filtrate and washings, *N,N'*-diphenylethylenediamine (254 mg, 1.2 mmol) was added and the

mixture was stirred for 40 minutes at 25 °C. The mixture was concentrated to dryness *in*

vacuo and the residue was dissolved in EtOAc (100 mL), which was washed with H$_2$O (3 x

30 mL), followed by saturated aqueous NaCl (30 mL). The separated organic layer was dried

(Na$_2$SO$_4$) and concentrated to dryness *in vacuo*. The residue was purified on a silica gel

column (by using neutralized silica gel; ICN Silica 63-200, 60 Å, 2.3 x 8 cm), eluted with 20-

33% EtOAc in hexane, to give <u>13</u> (321 mg, 67% as a white foam, recrystallized from

MeOH): mp 141-143 °C; MS *m/z* 405 (M⁺ - Ph); ¹H nmr (CDCl₃) δ 7.27-7.35 (m, 5H, C₆*H₅*),

6.89-6.80 (m, 6H, H-2, C₆*H₅*), 5.80 (d, 1H, H-5', $J_{4',5'}$ = 2.8 Hz), 5.79 (s, 1H, H-1'), 4.92 (m,

1H, H-2'), 4.61 (m, 2H, H-3', H-4'), 3.70 (s, 1H, C≡C*H*), 3.68 (m, 4H, NC*H₂*C*H₂*N), 1.30 and

1.45 (two s, 3H each, C*Me₂*). *Anal.* Calcd. for C₂₈H₂₇N₅O₃: C, 69.84; H, 5.65; N, 14.54.

Found: C, 69.72; H, 5.73; N, 14.42.

5-Ethynyl-1-[5-deoxy-5,5-(*N*,*N*'-diphenylethylenediamino)-2,3-*O*-isopropylidene-β-D-ribofuranosyl]imidazole-4-carboxamide (14).

A suspension of **13** (610 mg, 1.27 mmol) in NH₄OH-MeOH (5mL-10mL) containing H₂O₂

(30% w/v, 0.5 mL) was stirred for 7 hours at 25 °C. The mixture was concentrated to dryness

in vacuo and the residue was dissolved in EtOAc (100 mL), which was washed with H₂O (3

x 30 mL), followed by saturated aqueous NaCl (30 mL). The separated organic layer was

dried (Na₂SO₄) and concentrated to dryness *in vacuo*. The residue was purified on a silica gel

column (by using neutralized silica gel, 2.6 x 13 cm), eluted with 50-100% EtOAc in hexane,

to give **14** (491 mg, 78% as a white foam); MS *m/z* 499 (M⁺); ¹H nmr (CDCl₃) δ 7.29-7.35

(m, 5H, C₆*H₅*), 6.81-6.88 (m, 7H, H-2, amide proton, C₆*H₅*), 5.89 (d, 1H, H-5', $J_{4',5'}$ = 2.7 Hz),

5.80 (s, 1H, H-1'), 5.45 (br s, 1H, amide proton), 4.92 (m, 1H, H-2'), 4.67 (m, 1H, H-3'), 4.60

(m, 1H, H-4'), 3.68 (s, 1H, C≡C*H*), 3.68 (m, 4H, NC*H₂*C*H₂*N), 1.31 and 1.49 (two s, 3H

each, C*Me₂*).

5-Ethynyl-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide (16).

A solution of **14** (100 mg, 0.2 mmol) in a mixture of H₂O (2 mL) and THF (2 mL) was

stirred for 30 minutes at 25 °C in the presence of DIAION PK212 (Mitsubishi Kasei Co., H⁺

form, 2 mL as wet volume). The resin was filtered off and washed with hot MeOH. The

combined filtrate and washings were concentrated to dryness *in vacuo*. The residue **15** was

dissolved in MeOH (4 mL) and NaBH₄ (23 mg, 0.6 mmol) was added to the solution. The

mixture was stirred for 20 minutes at 25 °C. The mixture was concentrated to dryness *in*

vacuo and the residue was coevaporated three times with MeOH. The residue was purified on

a silica gel column (2.1 x 5 cm), eluted with 4-16% EtOH in CHCl₃, to give **16** (36 mg, 59%

as a colorless glass, recrystallized from EtOH-hexane): mp 145-146 °C; MS *m/z* 307 (M⁺); ¹H

nmr (CDCl₃) δ 7.82 (s, 1H, H-2), 6.95 (br s, 1H, amide proton), 5.96 (d, 1H, H-1', $J_{1',2'}$ = 3.3

Hz), 5.64 (br s, 1H, amide proton), 5.00 (dd, 1H, H-2', $J_{1',2'}$ = 3.3, $J_{2',3'}$ = 6.6 Hz), 4.94 (dd,

1H, H-3', $J_{2',3'}$ = 6.6, $J_{3',4'}$ = 3.3 Hz), 4.34 (ddd, 1H, H-4', $J_{3',4'}$ = $J_{4',5'a}$ = $J_{4',5'b}$ = 3.3 Hz), 3.93 (m,

1H, H-5'a), 3.86 (s, 1H, C≡C*H*), 3.81 (m, 1H, H-5'b), 2.80 (br s, 1H, 5'-OH), 1.37 and 1.61

(two s, 3H each, C*Me₂*). *Anal.* Calcd. for C₁₄H₁₇N₃O₅: C, 54.72; H, 5.58; N, 13.67. Found: C,

54.39; H, 5.55; N, 13.56.

5-Ethynyl-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (EICAR, 3).

An aqueous TFA solution (90% w/v, 2 mL) containing **16** (36 mg, 0.12 mmol) was stirred

for 10 minutes at 25 C. The solvent was removed *in vacuo* and the residue was coevaporated

several times with EtOH. The residue was purified on a silica gel column (1.7 x 2 cm), eluted

with 5-30% EtOH in CHCl₃, to give **3** (25 mg, 80% as a colorless glass). The physical data

for **3** has been reported previously (2).

5-Ethynyl-1-([5-³H]-β-D-ribofuranosyl)imidazole-4-carboxamide (18).

Wet DIAION PK212 ion-exchange resin (H⁺ form, about 0.2 mL), which was exposed to 5 N

HCl (10 mL) for 12 hours and washed with water until the water wash was neutral, was

added to a solution of **14** (12 mg, 0.024 mmol) in H₂O (0.2 mL) and THF (0.2 mL). This

mixture was stirred for 30 minutes at 25 °C, after which the solution was transferred using a

syringe, to a round bottom flask (5 mL volume). The solvent was removed *in vacuo*, the

residue obtained (**15**) was dissolved in MeOH (0.4 mL) and [³H]NaBH₄ (470 μg, 0.012

mmol, 99 mCi of specific activity 8.3 Ci/mmol) was added. The reaction was allowed to

proceed for 30 minutes with stirring at 25 °C, and the reaction mixture was applied to a

preparative silica gel plate which was developed using MeOH-CHCl₃ (15:85, v/v) as

development solvent. The TLC spot, having R_f 0.42 which was identical to that of unlabeled

17, was removed from the silica gel plate and EtOH-CHCl₃ (1:4, v/v; 10 mL) was added to

this silica gel fraction. Removal of the silica gel by filtration, washing the silica gel with

EtOH (2 x 2 mL) and removal of the solvent *in vacuo* from the combined filtrate and

washings afforded **17**. Addition of 90% TFA (0.6 mL) to the residue **17**, allowing the reaction to proceed for 15 minutes at 25 °C with stirring, and then removal of the solvent *in vacuo* gave a residue (**18**) which was dissolved in EtOH (0.5 mL). This solution was applied to one preparative silica gel TLC plate that was developed using MeOH-CHCl$_3$ (1:4, v/v). Removal of the silica gel spot having R$_f$ 0.22, which was identical to that of an unlabeled authentic reference standard, addition of EtOH (10 mL) to the silica gel fraction, removal of the silica gel by filtration, washing the silica gel with EtOH (2 x 2 mL) and removal of the solvent *in vacuo* from the combined filtrate and washings afforded the pure product **18** which was immediately formulated for storage as a solution in EtOH-H$_2$O (1:1, v/v; 1.5 mL). The radiochemical purity of **18** in this solution was determined by HPLC analysis and liquid scintillation counting. All of the radioactivity was associated with the eluting peak collected at 5.9 minutes (7.0 mCi total radioactivity, > 99% radiochemical purity), which was identical to the retention time of an authentic sample of unlabeled **18**. Quantitative HPLC analysis of the solution of [5-^{3}H]-**18** described above indicated that the chemical yield of [5-^{3}H]-**18** was 19% (1.2 mg) and that the specific acitivity was 1.56 Ci/mmol.

Acknowledgements

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