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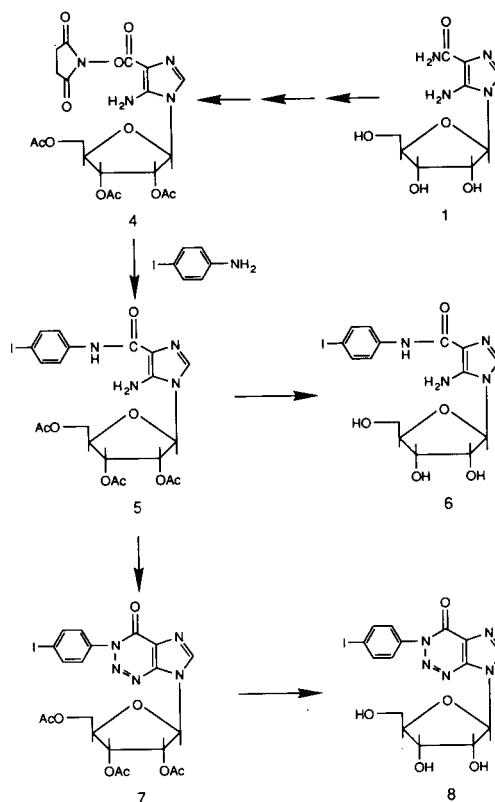
A model iodophenyl imidazole ribonucleoside has been synthesized to study biodistribution properties in laboratory animals. The key intermediate 5-amino-1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)imidazole-4-[*N*-(*p*-iodophenyl)carboxamide] (**5**) was synthesized by coupling *N*-succinimidyl-5-amino-1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)imidazole-4-carboxylate (**4**) and *p*-iodoaniline. Deacetylation of the intermediate compound gave 5-amino-1- β -D-ribofuranosylimidazole-4-[*N*-(*p*-iodophenyl)]carboxamide (**6**). Ring annulation *via* diazotization of **5** gave 7-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-[3-*N*-(*p*-iodophenyl)]-4-one (**7**). Subsequent deacetylation of **7** afforded 7- β -D-ribofuranosylimidazo[4,5-*d*]-*v*-triazin-[3-*N*-(*p*-iodophenyl)]-4-one (**8**). The radiolabeled compounds, [¹²⁵I]**5** and [¹²⁵I]**6** were prepared in a manner similar to the corresponding unlabeled compounds except that *p*-[¹²⁵I]iodoaniline was used for coupling with **4**. Biodistribution studies of iodine-125-labeled **5** and **6** were performed in female Fischer rats and tumor bearing nude mice. Compound **6** showed uptake in the brain and proliferating tissues such as tumor and bone-marrow.

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Introduction.

Radiolabeled nucleosides are useful nucleic acid precursors for the study of their incorporation into tumors as an indication of tumor activity [1]. Several radiolabeled 5-[¹⁸F]-fluorouridine analogues have been synthesized for measuring RNA synthesis and tumor growth rates *in vivo* [3-5]. It has been demonstrated in various cell systems that the ability of cells to transport nucleosides increases dramatically in a growing tumor [6-8]. The transport of nucleosides across the plasma membrane is mediated by specific, enzyme-like elements of the membrane. Through the action of kinases, nucleosides are metabolically "trapped" intracellularly in the form of 5'-phosphates [9-10]. The relative accumulation of several radiolabeled pyrimidine nucleosides in the tumor as compared to normal tissues has been evaluated *in vivo*. Tissue distribution studies of [methyl-¹⁴C]thymidine in normal and tumor bearing rats showed preferential accumulation of the radioactivity in the tumor with tumor/blood, tumor/prostate and tumor/muscle ratios of 3.09, 4.98 and 6.11, respectively [11]. Similarly, tissue distribution studies of 5-chloro-2'-deoxy-2'-fluoro[2-¹⁴C]uridine and 5-fluoro-2'-deoxy-2'-fluoro[2-¹⁴C]uridine in BDF₁ mice bearing a subcutaneous Lewis lung tumor showed high tumor/blood ratios [12].

The foregoing results motivated us to synthesize new radiolabeled nucleosides as potential biochemical tools to study phosphorylation and nucleic acid metabolism. Radiolabeled nucleosides due to their unique properties could serve as potential substrates for different enzymes of nucleic acid metabolism with subsequent incorporation (trapping) in tumors. The localization of radiolabeled nucleosides, or more importantly, their involvement in metabolic pathways and nucleic acid synthesis can serve as a probe for metabolism *in vivo*. Furthermore, their incorpo-



Scheme I

ration in the proliferating tissue could indicate early stages of tumor growth and provide an effective means for early tumor diagnosis.

The naturally occurring nucleotide, 5'-phosphate of 5-amino 1- β -D-ribofuranosylimidazole-4-carboxamide (AICA riboside, **1**) is an important precursor of *de novo* purine nucleotide biosynthesis [13]. A number of related nucleoside analogues such as 5-halo-1- β -D-ribofuranosyl-

imidazole-4-carboxamide [14], 2- β -D-ribofuranosylthiazole-4-carboxamide (tiazofurin) [15] and 2- β -D-ribofuranosylselenazole-4-carboxamide (selenazofurin) [16] show interesting antiviral [17] and antitumor [15,16] properties. Therefore, radiolabeling and biodistribution studies of this class of compounds are of biological interest. Since AICA riboside is readily available [18], the structural perturbations of this agent for radiolabeling and biodistribution evaluation have been pursued as a model for tiazofurin and selenazofurin. This preliminary report describes the synthesis, biodistribution properties and chemical transformation of 4-*N*-(*p*-iodophenyl) analogue of AICA riboside.

Results and Discussion.

Chemistry.

The synthesis of the succinimide activated ester, *N*-succinimidyl-5-amino-1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)imidazole-4-carboxylate (**4**), from the AICA riboside **1** via the synthesis of sodium 5-amino-1- β -D-ribofuranosylimidazole-4-carboxylate (**2**) and 5-amino-1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosylimidazole-4-carboxylic acid intermediate **3** has been reported by Srivastava *et al* [19]. However, difficulties are often encountered in the synthesis, work-up and isolation of the carboxylic acid precursor **3** in the crystalline form from a complex pyridine-acetic anhydride reaction mixture which must remain cold ($\sim 0^\circ$) for the entire reaction and work-up period. At ambient temperature in the presence of acetic anhydride, the carboxylic acid inter-

mediate **3** is amenable to ring annulation to yield 5-methyl-3-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)imidazo[4,5-*d*]-[1,3]oxazin-7-one [20] which is unstable [21] under the reaction conditions. In addition, the carboxylic acid **3** is also susceptible to decarboxylation and decomposition at acidic pH which can be encountered during the decomposition of excess acetic anhydride after removal of pyridine. These factors often contribute to the formation of a partly decomposed reaction mixture and low yields and also inhibit the crystallization of the carboxylic acid intermediate from the crude reaction mixture. In order to overcome this problem, we have devised a one-pot reaction as a modification of the literature procedure [19] for a convenient synthesis of **4**. The precursor, AICA riboside was hydrolyzed and acetylated [19] followed by coupling with *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide (DCC) to yield crystalline **4**.

Coupling of **4** with various amino acids and adamantanamine has been reported in the literature [19] to proceed smoothly at room temperature. However, an elevated temperature ($122^\circ \pm 3^\circ$) and relatively prolonged reaction period (3 hours) was required for the coupling of **4** with *p*-iodoaniline to yield the desired intermediate, 5-amino-1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)imidazole-4-*N*-(*p*-iodophenyl)carboxamide (**5**). The slow reaction could be attributed to the poor nucleophilic character of the amino group of *p*-iodoaniline as compared to the primary amine of amino acid esters or adamantanamine. Treatment of **5**

Table 1

Distribution of Radioactivity in Tissues of Female Fischer 344 Rats Following Intravenous Administration of [125 I]-**5** and of [125 I]-**6** [a]

Compound	Time after injection (minutes)	Mean percent injected dose/gm (range): tissue					
		Brain	Blood	Liver	Kidneys	Lungs	Thyroid
5	5	0.19 (0.18-0.20)	0.72 (0.70-0.76)	4.37 (4.13-4.56)	4.28 (4.21-4.34)	1.24 (1.17-1.27)	17.94 (17.26-18.66)
	30	0.09 (0.08-0.09)	0.41 (0.38-0.46)	2.01 (1.88-2.24)	2.35 (2.21-2.43)	0.70 (0.65-0.78)	16.72 (15.30-19.47)
	60	0.06 (0.06-0.07)	0.30 (0.29-0.37)	1.54 (1.52-1.58)	1.44 (1.31-1.61)	0.52 (0.49-0.57)	26.47 (23.09-31.02)
	120	0.06 (0.05-0.06)	0.22 (0.20-0.23)	1.16 (1.02-1.28)	0.88 (0.85-0.90)	0.36 (0.33-0.39)	37.45 (32.67-40.07)
	240	0.04 (0.04-0.05)	0.20 (0.19-0.22)	0.99 (0.96-1.04)	0.58 (0.55-0.61)	0.24 (0.23-0.25)	78.95 (51.88-131.62)
6	5	0.28 (0.25-0.32)	1.40 (1.13-1.74)	2.52 (2.34-2.82)	9.56 (7.52-11.85)	1.10 (0.96-1.28)	11.31 (9.44-12.66)
	30	0.14 (0.13-0.16)	0.46 (0.42-0.49)	1.79 (1.77-1.81)	2.17 (2.15-2.18)	0.43 (0.40-0.47)	6.45 (5.04-7.51)
	60	0.06 (0.05-0.07)	0.25 (0.23-0.27)	1.31 (1.21-1.41)	1.02 (0.97-1.09)	0.24 (0.23-0.25)	5.86 (5.46-6.22)
	240	0.01 (0.01-0.01)	0.10 (0.10-0.10)	0.32 (0.29-0.35)	0.19 (0.18-0.20)	0.08 (0.08-0.09)	17.41 (12.30-24.87)

[a] Each animal (three animals per time point) received either 0.43 mCi of [125 I]-**5** or 1.05 mCi of [125 I]-**6** by tail vein injection.

Table 2

Distribution of Radioactivity in Tissues of Colorectal Carcinoma Implanted Nude Mice Following Intravenous Administration of [¹²⁵I]-**6** [a]

Compound	Time after injection (minutes)	Mean percent injected dose/gm (range): tissue				
		Brain	Blood	Liver	Kidneys	Lungs
6	30	0.18 (0.09-0.28)	1.12 (0.52-1.72)	6.07 (1.98-10.17)	3.82 (1.85-5.79)	2.95 (0.96-4.94)
	240	0.10 (0.08-0.11)	0.89 (0.88-0.91)	5.88 (5.69-6.07)	2.47 (2.00-2.94)	2.06 (1.94-2.18)
	1440	0.03 (0.02-0.03)	0.10 (0.08-0.11)	0.73 (0.58-0.88)	0.35 (0.28-0.42)	0.41 (0.34-0.48)

Compound **6** (continued)

Time after injection (minutes)	Small Intestine	Large Intestine	Spleen	Muscle	Marrow	Tumor
30	1.98 (1.05-2.90)	1.43 (0.61-2.25)	0.96 (0.46-1.45)	1.44 (0.21-2.66)	1.53 (0.72-2.35)	0.74 (0.43-1.06)
240	2.71 (2.67-2.76)	1.35 (1.23-1.46)	0.72 (0.63-0.82)	0.33 (0.29-0.37)	1.49 (1.32-1.65)	0.71 (0.68-0.74)
1440	0.16 (0.12-0.20)	0.13 (0.12-0.15)	0.12 (0.11-0.13)	0.09 (0.08-0.10)	0.89 (0.73-1.05)	0.10 (0.08-0.13)

[a] Each animal (two animals per time point) received 2.9 mCi [¹²⁵I]-**6** by tail vein injection.

with ammonium hydroxide provided the deacetylated product, 5-amino-1- β -D-ribofuranosylimidazole-4- N -(*p*-iodophenyl)carboxamide (**6**). The diazotization of AICA riboside gives 2-azainosine which shows cytotoxic [22] properties. Similarly, the diazotization of **5** followed by intramolecular diazocoupling gave the ring annulated compound, 7-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-[3-*N*-(*p*-iodophenyl)]4-one (**7**). Deacetylation of **7** using ammonium hydroxide provided the 3-*N*-(*p*-iodophenyl)-2-azainosine analogue, 7- β -D-ribofuranosylimidazo[4,5-*d*]-*v*-triazin-[3-*N*-(*p*-iodophenyl)]4-one (**8**).

Biological Studies.

The biodistribution of the model radiolabeled imidazole nucleoside, [¹²⁵I]**6** was evaluated in normal rats (Table 1) and tumor implanted nude mice (Table 2). The agent shows initial brain uptake both in normal rats and nude mice indicating the ability of this agent to cross the blood-brain barrier. Fairly constant levels (5.86%-17.41% dose/gm) of radioactivity over a prolonged period in the thyroid were observed indicating *in vivo* stability of the radiolabel. In the nude mice, the agent showed moderate initial tumor uptake, although the uptake in the other proliferating tissues such as in the bone-marrow and intestine was approximately twice as high. Compound [¹²⁵I]**5**, the triacetyl derivative of [¹²⁵I]**6**, showed relatively low brain

uptake and higher thyroid uptake as compared to the parent nucleoside in rats.

Summary and Conclusion.

Radiolabeled nucleosides are important as potential tools to study biochemical pathways and metabolism of nucleic acids. The identification of radiolabeled nucleosides that show high tumor uptake relative to other proliferating tissues is of further interest because such agents could potentially be used as tumor marker agents. The synthesis and radiolabeling of AICA riboside demonstrate that iodine can be stabilized as an *N*-(*p*-iodophenyl)carboxamide on similar nucleosides. The introduction of iodo-phenyl group to a carboxamide may also represent an interesting structural perturbation making the nucleoside more lipophilic and penetrable to the blood-brain barrier [23]. Similar structural modifications for radiolabeling of antitumor nucleosides, such as tiazofurin and selenazofurin, will be evaluated for their tumor uptake relative to other tissues and penetration across the blood-brain barrier *in vivo*.

EXPERIMENTAL

General Procedures.

Melting points were determined in capillary tubes on a Buchi SMP-20 apparatus and are uncorrected. The thin-layer chromatographic analyses

(tlc) were performed using 250 μ m-thick layers of silica gel GF coated on glass plates (Analtech, Inc.). The proton nuclear magnetic resonance spectra (nmr) were obtained at 60 MHz with a Varian EM360A instrument. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

Animal Tissue Distribution Studies.

The distribution of radioactivity was determined in normal 14-18 week-old female Fisher rats (163-189 g) and female athymic nude mice (20.0-26.5 g) implanted with SW 948 colorectal carcinoma, made available from the Oak Ridge Associated Universities through the courtesy of Dr. L. C. Washburn. The animals were allowed food and water ad libitum before and during the course of the experiment. The radioiodinated compound **5** was formulated in 10% dimethylsulfoxide (DMSO) and saline. The radioiodinated compound **6** was formulated in saline. The solutions were filtered through a 0.22 μ m Millipore filter and injected *via* a lateral tail vein into the ether-anesthetized animals. After the times indicated, the animals were killed by cervical fracture, and blood samples obtained by cardiac puncture. The organs were then removed, rinsed with saline solution, and blotted dry to remove residual blood. The organs were weighed (Mettler AE 163) and counted in a sodium iodide autogamma counter (Packard Instruments). Samples of the injected radioactive solutions were also assayed as standards to calculate the percent injected dose per gram of tissue values. The weight of the rat thyroid glands was calculated by multiplying the animal weight by (7.5 mg/100 g).

N-Succinimidyl-5-amino-1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)imidazole-4-carboxylate (**4**).

A sample of sodium 5-amino-1- β -D-ribofuranosylimidazole-4-carboxylate (**2**, 2.33 g, 8.3 mmoles) was prepared as reported earlier [19] and suspended in anhydrous pyridine (60 ml) with stirring at 0°. Acetic anhydride (27 ml) was added dropwise over a period of 15 minutes to the stirred suspension at 0°. After addition, the mixture was stirred for 5 hours at 0°. Fuller's earth was added to the reaction mixture, stirred for 10-15 minutes and filtered through a pad of Fuller's earth. The filtrate was evaporated under vacuum to form a syrup. A small aliquot of this syrup was crystallized [19] to give **3**, mp 143-144° (lit [19] 145°). In a typical preparation, dimethylformamide (10 ml) was added and evaporated under vacuum to remove water. The solvents were evaporated at an ambient temperature (not exceeding 25°) under vacuum. The crude compound **3** obtained as a brown syrup was used for the synthesis of **4**.

Dicyclohexylcarbodiimide (2.06 g, 10 mmoles) and *N*-hydroxysuccinimide (1.15 g, 10 mmoles) were added to a solution of **3** in dimethylformamide (25 ml). The reaction mixture was stirred overnight at room temperature. Acetic acid (5 ml) was added and the mixture stirred for 5 hours. The crystalline dicyclohexylurea was removed by filtration and the filtrate was evaporated, diluted with water and extracted twice with ethyl acetate (50 ml). The ethyl acetate portion was dried (sodium sulfate) and evaporated under vacuum to a syrup (55-60°). The compound was purified by column chromatography using silica gel (Davison) packed in chloroform. The compound was charged and eluted with chloroform and fractions (20-40 ml each) were collected. The fractions containing pure product (tlc, 2% methanol in ethyl acetate) were combined and evaporated under vacuum. The crude compound (syrup) was crystallized from boiling benzene to provide **4** in 20% (819 mg) overall yield, mp 111° (lit [19] mp 110°).

5-Amino-1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)imidazole-4-[*N*-(*p*-iodophenyl)-carboxamide] (**5**).

A solution of *p*-iodoaniline (45 mg, 0.2 mmole) and **4** (97 mg, 0.2 mmole) in dimethylformamide (1 ml) was heated (bath temperature 105°) with stirring overnight. Analysis by tlc in 50% ethyl acetate in chloroform indicated complete reaction. The reaction mixture was removed from the oil bath to cool and evaporated. The product was isolated by silica gel column chromatography using chloroform as the solvent. The desired fractions were combined and evaporated to yield 92

mg (78%) of **5** as a syrup; nmr (deuteriochloroform): δ 8.65 (bS, 1, CONH), 7.42-7.8 (q, 4, A₂B₂, J = 4 Hz and 10 Hz, phenyl), 7.27 (S, 1, H₂), 5.2-5.9 (m, 5, H_{1'}, H_{2'}, H_{3'}, and NH₂), 4.48 (S, 3, H_{4'} and H_{5'}), 2.15 (S, 9, 3 x acetyls).

Anal. Calcd. for C₂₁H₂₃IN₄O₈: C, 43.02; H, 3.95; N, 9.56; I, 21.64. Found: C, 43.24; H, 4.32; N, 9.24; I, 21.86.

5-Amino-1- β -D-ribofuranosylimidazole-4-[*N*-(*p*-iodophenyl)carboxamide] (**6**).

Methanol (1 ml) and ammonium hydroxide (0.5 ml) were added to **5** (116 mg, 0.2 mmole) and stirred at room temperature overnight. The product crystallized out of the reaction mixture after the vessel was scratched. The crystalline compound was rinsed with ethanol and recrystallized (ethanol) to yield 68 mg (74%) of **6**, mp 230-232° (shrinks 202-206°).

Anal. Calcd. for C₁₅H₁₇IN₄O₈: C, 39.14; H, 3.72; N, 12.17; I, 27.58. Found: C, 39.11; H, 3.68; N, 11.72; I, 27.56.

7-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)imidazo-[4,5-*d*]-*v*-triazin-[3*N*-(*p*-iodophenyl)]-4-one (**7**).

A solution of **5** (254 mg, 0.43 mmole) in methanol (0.5 ml) in a 100 ml round-bottom flask was cooled and stirred in a dry ice/2-propanol bath (-20° to -40°). Hydrochloric acid (6 *N*, 2.5 ml) was cooled and added to the mixture. An aqueous solution of sodium nitrite (105 mg, 1.5 mmoles) was added slowly to the above mixture which was stirred for 20 minutes at -25° to -40°. Ethyl acetate (25 ml) was added and the solution was adjusted to pH 6-7 by adding concentrated sodium hydroxide while maintaining the temperature below -25°. The ethyl acetate layer was separated and the aqueous layer extracted with additional ethyl acetate. The ethyl acetate portions were combined and washed with a sodium bicarbonate solution followed by a sodium chloride solution. The ethyl acetate portion was dried (sodium sulfate) and evaporated. The residue was passed through a column packed with Davison silica gel slurry in chloroform; eluted with a chloroform:ethyl acetate (8:6, v/v) solution. The product appeared in fractions 12-19 which were combined and evaporated to yield (190 mg, 74%) of **7** as a foam which was characterized by nmr (deuteriochloroform): δ 8.42 (s, 1, H₆), 7.32-8.08 (g, 4, A₂B₂, J = 22 Hz and 9 Hz, Phenyl), 6.4 (d, 1, J = 5 Hz, H_{1'}), 5.9 and 5.67 (m and m, 1 and 1, H_{2'} and H_{3'}), 4.47 (s, 3, H_{4'} and H_{5'}), 2.1 (s, 9, 3 x acetyls) and analyzed as the corresponding deacetylated product **8** described below.

7- β -D-Ribofuranosylimidazo[4,5-*d*]-*v*-triazin-[3*N*-(*p*-iodophenyl)]-4-one (**8**).

Ammonium hydroxide (1 ml) was added to a solution of **7** (180 mg, 0.3 mmole) in methanol (2 ml) and stirred at room temperature overnight, and the solvent was evaporated under vacuum. The residue was triturated with water to yield a crystalline product which was collected by filtration. The crude compound was recrystallized from methanol and water, filtered, and dried to yield (126 mg, 80%) of **8** as a brown colored solid, mp 158° (shrinks 115-119°).

Anal. Calcd. for C₁₅H₁₄IN₄O₈: C, 36.16; H, 3.43; N, 14.06; I, 25.47. Found: C, 36.36; H, 3.36; N, 13.96; I, 25.83.

Radiochemical Synthesis of [¹²⁵I]**5** and [¹²⁵I]**6**.

The *p*-[¹²⁵I]iodoaniline (1 mCi, specific activity 375 mCi/mmmole) was prepared as reported earlier [23] and dissolved in anhydrous dimethylformamide (0.5 ml). Ester **4** (12 mg, 0.025 mmole) was added and heated (bath temperature 105 to 110°) with stirring for 4 hours. The reaction mixture was allowed to gain room temperature and applied on a tlc plate (20 x 20 cm, 250 micron thickness) which was developed in chloroform:ethyl acetate (1:1, v/v). The radioactive band corresponding to **5** was scraped and eluted with ethyl acetate which after evaporation under argon gave 215 μ Ci (21.5% radiochemical yield) of [¹²⁵I]**5**. A solution of [¹²⁵I]**5** (145 μ Ci) in methanol (1 ml) and ammonium hydroxide (0.5 ml) was stirred for 1 hour. The solvent was evaporated under argon to a reduced volume (0.25 ml) and applied on a tlc plate (20 x 20 cm, 250 mm thickness) which was developed in ethyl acetate. The band corresponding to **6** was scraped and eluted with ethyl acetate:2-propanol:water (7:1:2, v/v, top layer) to provide 64 μ Ci (44% radiochemical yield) of [¹²⁵I]**6**. The radiolabeled agent, [¹²⁵I]**5** and [¹²⁵I]**6**, moved identical to the correspond-

ing unlabeled standards and were homogenous on tlc when examined under uv light and by radioactive scanning.

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