HETEROCYCLES, Vol. 76, No. 2, 2008, pp. 1329 - 1335. © The Japan Institute of Heterocyclic Chemistry
Received, 7th April, 2008, Accepted, 28th May, 2008, Published online, 2nd June, 2008. COM-08-S(N)99

A CONVENIENT SYNTHESIS OF OPTICALLY ACTIVE BIOPTERIN

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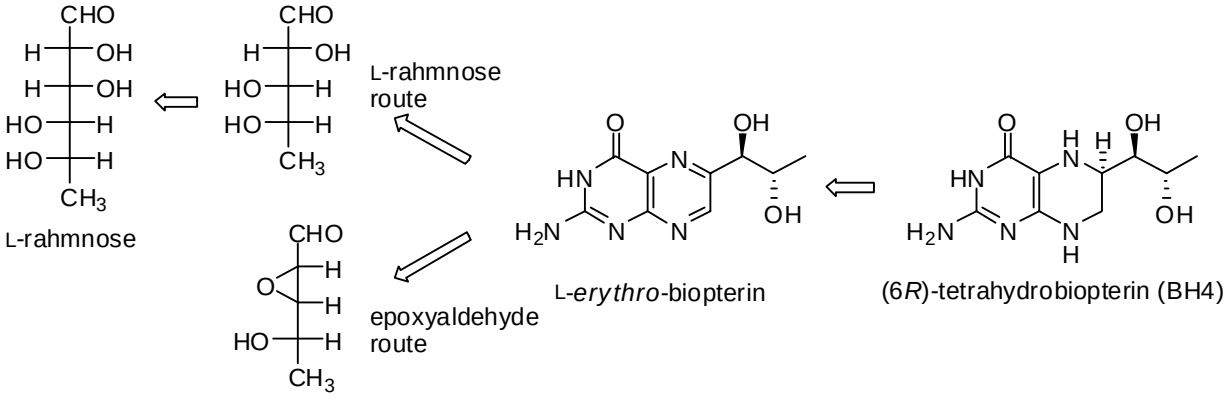
Abstract – Naturally occurring L-*erythro*-biopterin is synthesized using regioselective pteridine-ring formation from the chiral α,β-epoxyaldehyde intermediate which is prepared from ethyl L-lactate via Sharpless epoxidation.

INTRODUCTION

Biopterin, L-*erythro*-biopterin, is one of the most common naturally occurring pteridines and is found in a wide range of biological samples. Its tetrahydro derivative, that is (6*R*)-tetrahydrobiopterin (BH4), is an important cofactor for the metabolism of aromatic amino acids and the biosynthesis of catecholamines and nitrogen oxide (NO). BH4 deficiency in a human body is known to contribute to various diseases such as malignant hyperphenylalanemia and DOPA (3,4-dihydroxyphenylalanine) responsive dystonia.[1] For the chemotherapeutic treatement of such diseases, the practical synthesis of BH4 has been studied for a long time, and recently the requirement for BH4 increased because of enlargement of its pharmaceutical efficacy. Since catalytic hydrogenation of L-*erythro*-biopterin to BH4 succeeded to produce the asymmetric carbon on the heterocyclic part,[2] the enantioselective preparation of the dihydroxypropyl side chain has remained the largest remaining problem. So far, L-*erythro*-biopterin for practical use has been

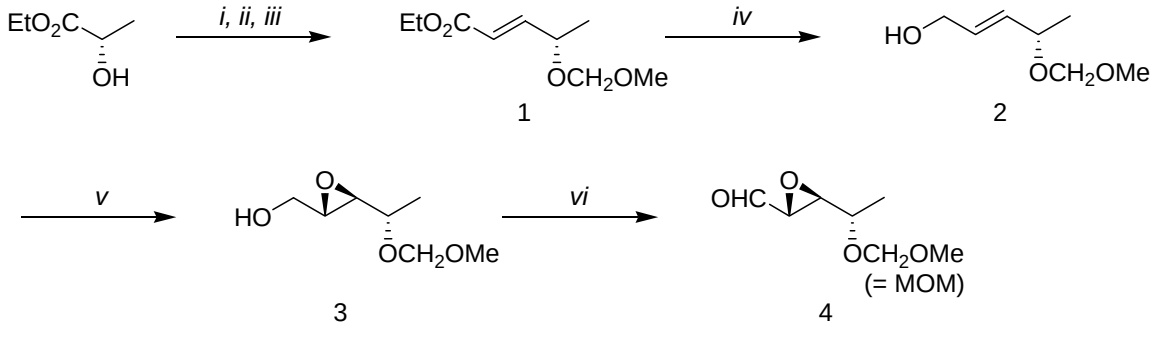


Scheme 1.Retrosynthetic routes of optically active BH4.

synthesized from commercially available L-rahmnose, and the retrosynthetic route is illustrated as L-rahmnose route in Scheme 1.[3] However, there are several problems in the large-scale transformation of L-*erythro*-biopterin from the sugar, and the most serious one is the use of large amounts of dangerous chemicals such as ethanthiol and *m*-chloroperoxybenzoic acid. In contrast to that, we have reported regioselective pterin-forming cyclization of α,β-epoxyaldehyde, and in that paper we demonstrated the application for L-*erythro*-biopterin synthesis independent of naturally occurring sugar.[4] Unfortunately, because the previous synthesis[4] needed optical resolution of the epoxyaldehyde precursor as well as a long synthetic pathway, the method shown as epoxyaldehyde route in Scheme 1 did not attract an interest for long period. Described herein are the enantioselective preparation of an optically active α,β-epoxyaldehyde precursor and the improved synthesis of naturally occurring L-*erythro*-biopterin.

RESULTS AND DISCUSSION

Preparation of the α,β-epoxyaldehyde precursor (**4**) is shown in Scheme 2. The hydroxy group of ethyl (*S*)-2-hydroxypropionate was protected (92% yield) by a methoxymethyl group (MOM), and the ester group was converted to an aldehyde by the action of diisobutylaluminum hydride (DIBAL). Horner-Emmons C=C bond extension[5] of the aldehyde yielded the α,β-unsaturated ester **1** in 82% yield (2 steps). The ester group was reduced to an alcohol (89% yield), and the allylic alcohol **2** was converted to chiral epoxide **3** in 97% yield and 99% *de* by the treatment with (+)-diisopropyl tartrate, tetraisopropoxytitanium, and cumene hydroperoxide (Sharpless asymmetric epoxidation[6]). Finally, the chiral epoxide was oxidized to the α,β-epoxyaldehyde precursor (**4**) by the action of DMSO, oxalyl chloride, and triethylamine (Swern oxidation[7]) in 70% (overall 46%) yield. During the transformations, epimerization of asymmetric carbons was not detected. Due to instability, the key precursor **4** was used immediately to the next pteridine-ring formation. Contrary, the overall yield of the optically active α,β-epoxyaldehyde precursor was less than 5% in the previous method. [4]

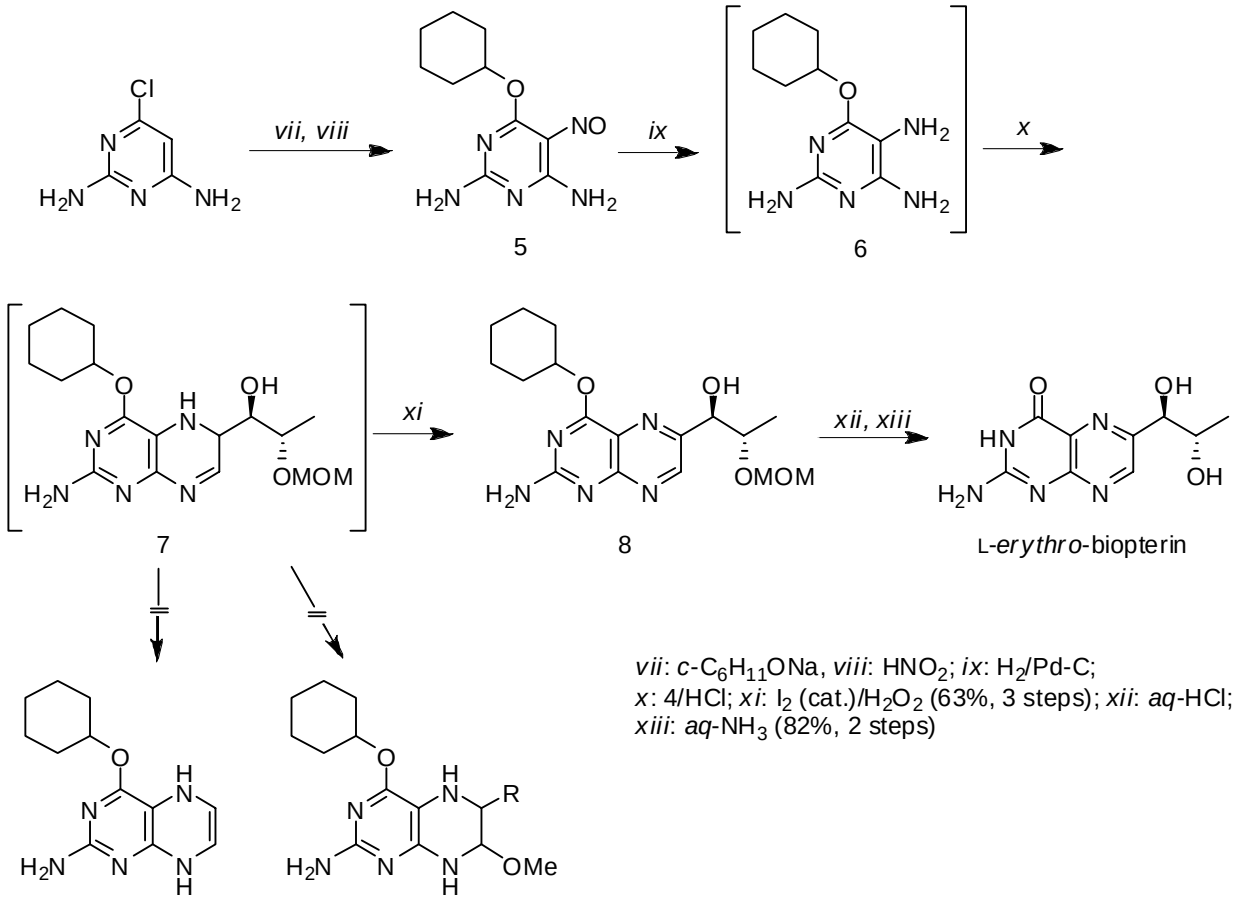


i: $MeOCH_2Cl$ (92%); *ii*: DIBAL; *iii*: $(EtO)_2P(O)CH_2CO_2Et$ (82% 2 steps);
iv: $LiAlH_4$ (89%); *v*: Sharpless epoxidation (97%); *vi*: Swern oxidation (70%)

Scheme 2. Synthesis of optically active α,β-epoxyaldehyde precursor of biopterin.

2,4-Diamino-5-nitrosopyrimidine derivative **5**, the other key precursor, was prepared from 2,4-diamino-6-chloropyrimidine in 89% yield (2 steps) as shown in Scheme 3. Because of instability of the intermediates **6** and **7**, the following 3 steps were carried out sequentially without isolation of the products. Catalytic hydrogenation of **5** on 5% Pd-C in acetonitrile gave triaminopyrimidine **6**, and this was immediately used to the pteridine-forming cyclocondensation with **4** in the presence of a stoichiometric amount of HCl. The next oxidation of the resulting 7,8-dihydropterin intermediate **7** was carried out by using an excess amount of hydrogen peroxide in the presence of a catalytic amount of iodine (10 %), and the biopterin derivative **8** with protecting groups was obtained in 63% overall yield. The compound **8** is soluble in common organic solvents and can be purified by silica gel column chromatography. Deprotection of **8** was performed by sequential treatment with aq. HCl and aq. NH_3 in 82% overall yield, and L-*erythro*-biopterin was fully characterized by HPLC in the comparison with an authentic sample.[1,3]

In the previous pteridine-ring formation,[4] the reaction was carried out in the presence of excess amounts of formic acid and iodine in methanol, and the yield of biopterin derivative was 30% at most. One equivalent of stronger acidic HCl was used to prevent a retroaldol-type side chain cleavage reaction of dihydropteridine intermediate **7**, and the side reaction is known to be accelerated under basic conditions.[8] The less nucleophilic solvent, acetonitrile, was chosen as the solvent instead of methanol, which afforded



Scheme 3. Synthesis of biopterin and side reactions.

undesired methanol-adduct with **7**.[8]

EXPERIMENTAL

Methoxymethyl ether of (-)-ethyl (S)-2-hydroxypropionate. A mixture of (-)-ethyl tartrate (24.0 g, 0.203 mol), ethyldiisopropylamine (39.4 g, 0.305 mol), and chloromethoxymethane (24.5 g, 0.304 mol) in DMF (120 mL) was stirred at 50 °C for 15 h. To this was added water (120 mL), and the mixture was extracted with a 1:1 (v/v) mixture of hexane and Et_2O (3 times, total 360 mL). The organic extracts were washed 3 times with 15% aqueous NaCl solution and dried over $MgSO_4$ (24 g). The solution was stirred with silica gel (12 g) for 30 min, and solvent was removed by evaporation. The titled compound (28.7 g) was obtained as colorless oil. ^{1}H NMR ($CDCl_3$): δ/ppm = 1.29 (t, 3H, *J* = 7.2 Hz), 1.43 (d, 3H, *J* = 7.2 Hz), 3.39 (s, 3H), 4.20 (q, 2H, *J* = 7.2 Hz), 4.22 (q, 1H, *J* = 7.2 Hz), 4.68 (d, 1H, *J* = 7.2 Hz), 4.71 (d, 1H, *J* = 7.2 Hz).

*Ethyl (S)-4-methoxymethoxy-2-pentenoate (**1**).* To a solution of methoxymethyl ether of (-)-ethyl (*S*)-2-hydroxypropionate (28.6 g, 0.176 mol) in Et_2O (290 mL) was added 0.98 M toluene solution of diisobutylaluminium hydride (DIBAL) (216 mL, 0.212 mol) at -78 °C. After 5 min, to this was added a 1:1(v/v) mixture of water and MeOH (140 mL), and the mixture was stirred at rt for 1 h. Precipitates were removed by filtration and, the organic filtrate was washed 2 times with saturated aqueous NaCl solution (100 mL). The combined water layer was extracted with Et_2O (100 mL x 3), and combined organic solution was dried over $MgSO_4$. The solution was stirred with silica gel (8.6 g), and solvents were removed by evaporation. The resulting colorless oil was subjected to the following reaction. To a mixture of LiCl (8.22 g, 0.176 mol), triethyl phosphonoacetate (39.5 g, 0.176 mol), and DBU (26.8 g, 0.176 mol) in MeCN (290 mL) was added slowly the full amount of the intermediate. After 5 min to this was added water (290 mL), and the mixture was extracted with Et_2O (290 mL x 2). The combined extracts were washed with 10% aqueous NaCl solution and dried over $MgSO_4$. Compound **1** (24.9 g, 75% for 2 steps) was obtained as pale yellow oil after concentration. ^{1}H NMR ($CDCl_3$): δ/ppm = 1.30 (t, 3H, *J* = 7.2 Hz), 1.31 (d, 3H, *J* = 6.8 Hz), 3.55 (s, 3H), 4.20 (q, 2H, *J* = 7.2 Hz), 4.36 (d.d.q, 1H, *J* = 1.6, 6.0, 6.8 Hz), 4.64 (s, 2H), 5.99 (d.d, 1H, *J* = 1.6, 15.6 Hz), 6.85 (d.d, 1H, *J* = 6.0, 15.6 Hz); IR (film): ν/cm^{-1} = 2980 (C-H), 1720 (C=O), 1298, 1271, 1080, 1035, 918, 866; Anal. Calcd for $C_9H_{16}O_4$: C, 57.43; H, 8.57. Found: C, 57.25; H, 8.46.

*(S)-4-Methoxymethoxy-2-penten-1-ol (**2**).* To a suspension of $LiAlH_4$ (7.5 g, 0.198 mol) in Et_2O was added $AlCl_3$ (8.78 g, 0.066 mol) at 0 °C, and the mixture was stirred for 15 min. A solution of **1** (24.8 g, 0.066 mol) in Et_2O was added slowly at 0 °C, and the reaction mixture was quenched by the addition of water (50 mL). After stirring for 1 h at rt, the mixture was filtered and washed with water (50 mL) followed by saturated aqueous NaCl solution (50 mL). The water solutions were extracted 3 times with

Et_2O (75 mL), and combined organic solutions were dried over $MgSO_4$. After concentration, the crude oil was subjected to column chromatography on silica gel during which it was eluted with mixtures of hexane and EtOAc (4:1 and 2:1), and **2** (17.1 g, 89%) was obtained as colorless oil. ^{1}H NMR ($CDCl_3$): δ/ppm = 1.28 (d, 3H, *J* = 6.8 Hz), 3.37 (s, 3H), 4.16 (d.d, 2H, *J* = 1.6, 5.2 Hz), 4.22 (d.q, 1H, *J* = 6.8 Hz), 4.58 (d, 1H, *J* = 6.8 Hz), 4.68 (d, 1H, *J* = 6.8 Hz), 5.64 (d.d.t, 1H, *J* = 1.6, 6.8, 15.6 Hz), 5.83 (d.t, 1H, *J* = 5.2, 15.6 Hz). ν/cm^{-1} = 3250 (O-H), 2930 (C-H) 1375, 1216, 1159, 1097, 1032, 917; Anal. Calcd for $C_7H_{14}O_3$: C, 57.51; H, 9.65. Found: C, 57.01; H, 9.39.

*(2R,3R,4S)-2,3-epoxy-4-methoxymethoxypentan-1-ol (**3**).* To a mixture of (+)-diisopropyl tartrate (2.98 g, 12.7 mmol), tetraisopropoxy titanium (3.01 g, 10.6 mmol), and molecular sieves 4A (7.75 g) in CH_2Cl_2 (155 mL) were added 80% cumene hydroperoxide (60.5 g, 0.318 mol) and **2** (15.5 g, 0.106 mol) at 0 °C. The mixture was stirred for 18 h, and then water (16 mL) was added and the mixture was filtrated and dried over Na_2SO_4. Column chromatography on silica gel eluting with mixtures of hexane and EtOAc (10:1 and 3:1) gave **3** (16.7 g, 97%). ^{1}H NMR ($CDCl_3$): δ/ppm = 1.94 (d, 3H, *J* = 6.8 Hz), 2.93 (d.d, 1H, *J* = 2.5, 5.6 Hz), 3.13 – 3.16 (m, 1H), 3.38 (s, 3H), 3.59 (d.q, 1H, *J* = 5.6, 6.8 Hz), 3.70 (d.d, 1H, *J* = 4.0, 12.4 Hz), 4.67 (d, 1H, *J* = 7.2 Hz), 4.69 (d, 1H, *J* = 7.2 Hz). IR (film): ν/cm^{-1} = 2930 (C-H), 1102, 1034, 914. Anal. Calcd for $C_7H_{14}O_4$: C, 51.84; H, 8.70; Found: C, 52.07; H, 8.58.

*(2*R,*3*R,*4*S*)-2,3-epoxy-4-methoxymethoxypentanal (**4**).* To a solution of DMSO (5.85 g, 74.9 mmol) in CH_2Cl_2 (150 mL) was added oxaryl chloride (4.70 g, 37.0 mmol) at - 60 °C, and after 30 min a solution of **3** (1.50 g, 9.25 mmol) in CH_2Cl_2 (30 mL). The mixture was stirred for 30 min, and triethylamine (15.0 g, 148 mmol) was added. The reaction was quenched by the addition of water (30 mL). The organic solution was separated, and the aqueous solution was extracted with CH_2Cl_2 (50 mL x 3). Combined organic solution was dried over Na_2SO_4 and concentrated. Column chromatography on silica gel eluting with a mixture of hexane and EtOAc (3:1) gave **4** (1.15 g, 78%). IR (film): ν/cm^{-1} = 2710 (C-H), 1690 (C=O), 1280, 1175, 1080, 1020, 920; ^{1}H NMR ($CDCl_3$): δ/ppm = 1.31 (d, 3H, *J* =6.4 Hz), 3.24 (d.d, 1H, *J* = 2.0, 4.4 Hz), 3.35 (s, 3H), 3.40 (d.d, 1H, *J* = 2.0, 6.4 Hz), 3.77 (d.q, *J* = 4.4, 6.4 Hz), 4.63 (d, 1H, *J* = 7.2 Hz), 4.65 (d, 1H, *J* = 7.2 Hz), 9.07 (d, 1H, *J* = 6.4 Hz).

*2,6-Diamino-4-cyclohexyloxy-5-nitrosopyrimidine (**5**).* To boiling cyclohexanol (340 mL) was added 60% NaH (12.2 g, 0.39 mol) slowly, and, then, 2,4-diamino-6-chloropyrimidine (22.0 g, 0.15 mol) was added. After refluxing for 2 h, the mixture was concentrated by a rotary evaporator. To this were added water (75 mL) and $NaNO_2$ (10.9 g, 0.16 mol), and the mixture was acidified to pH 5 by acetic acid. Filtration of the resulting reddish purple precipitates followed by washing with water and Et_2O gave **5** (27.9 g, 89%). Mp 260 °C (decomp); ^{1}H NMR (DMSO-d_6): δ/ppm = 1.34 (m, 3H), 1.58 (m, 3H), 1.75 (m, 2H), 2.00 (m, 2H), 5.31 (m, 1H), 7.72 (d, 2H, *J* = 3.6 Hz), 7.94 (d, 1H, *J* = 4.4 Hz), 10.09 (d, 1H, *J* = 4.4 Hz).

*Protected biopterin: compound **8**.* A suspension of **5** (88.9 mg, 0.38 mmol) and 5% Pd-C (8.9 mg) in

MeCN (2.2 mL) was shaken under H_2 atomosphere at rt until all of the **5** dissolved. The mixture was filtered, and to this were added 3 M HCl (0.1 mL) and **4** (50 mg, 0.31 mmol). After stirring for 1 h, to this were added I_2 (7.9 mg, 0.03 mmol) and 30% H_2O_2 (0.18 mL, 1.6 mmol), and the mixture was stirred for 12 h. A saturated aqueous solution of Na_2SO_3 (1 mL) was added, and solvents were removed by evaporation. The residue was extracted with $CHCl_3$ (1 mL x 3), and the extracts were concentrated. Silica gel column chromatography eluting with a mixture of EtOAc and MeOH (50:1) gave **8** (71.3 mg, 63%) as yellow solid. Mp 76 – 79 °C; ^{1}H NMR ($CDCl_3$): δ/ppm = 1.25 (d, 3H, *J* = 6.0 Hz), 1.30 – 1.50 (m, 3H), 1.61 – 1.74 (m, 3H), 1.83 – 1.90 (m, 2H), 2.05 – 2.14 (m, 2H), 3.28 (s, 3H), 3.99 (d.q, 1H, *J* = 5.2, 6.0 Hz), 4.61 (d, 1H, *J* = 7.0 Hz), 4.70 (d, 1H, *J* = 7.0 Hz), 4.90 (d, 1H, *J* = 5.2 Hz), 5.30 (t.t, 1H, *J* = 4.0, 5.6 Hz), 5.88 (br-s, 2H), 8.96 (s, 1H). IR (film): ν/cm^{-1} = 3331 (O-H), 2935 (C-H), 1595, 1457, 1099, 1031, 918. HRMS Calcd for $C_{17}H_{25}N_5O_4$: m/z = 363.1907. Found: m/z = 363.1909.

L-erythro-*Biopterin*.[3,4] To a solution of **8** (445 mg, 1.23 mmol) in MeOH (1 mL) was added 3 M HCl (5 mL), and the mixture was stirred at 50 °C for 23 h. The solution was neutralized (pH 7) by the addition of a 28% NH_4OH solution. Separation of the resulting yellow precipitates followed by washing with water gave title compound (240 mg, 82%). HPLC (Column: Inertsil® ODS-3, Eluant: 5% MeOH in 0.1 M phosphate buffer (pH 3.0)) of the product was identical with that reported in the reference.[1] IR (nujol): ν/cm^{-1} = 3450, 3200, 1693, 1465, 1380, 1300. ^{1}H NMR (CF_3COOD): δ/ppm = 1.53 (d, 3H, *J* = 6.4 Hz), 4.69 (d.q, *J* = 6.4 and 3.6 Hz, 1H), 5.43 (d, 1H, *J* = 3.6 Hz), 9.23 (s, 1H); UV (0.1 M aqueous HCl) λ_{max}/nm (ε): 322 (8000). CD (pH 5.3 phosphate buffer):[9] λ_{max}/nm: 330 (-), 292 (+), 248 (-), 224 (+).

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