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New ways to derivatize at position 6 of 7,7-dimethyl-7,8-dihydropterin

Genbin Shi and **Xinhua Ji***
Biomolecular Structure Section, Macromolecular Crystallography Laboratory, National Cancer Institute, 1050 Boyles Street, Frederick, MD 21702, USA

Abstract

Reported are the synthesis of two intermediates for derivatization at position 6 of 7,7-dimethyl-7,8-dihydropterin: 6-carboxylic acid ethyl ester-7,7-dimethyl-7,8-dihydropterin, which is a novel compound, and 6-aldehyde-7,7-dimethyl-7,8-dihydropterin, which is synthesized by a new method with a yield of 90%.

Keywords

Pterin; Carboxylation; Bromination; Ester; Aldehyde

The folate biosynthetic pathway is essential for bacterial growth. Targeting key enzymes in the pathway, sulfonamides and diaminopyrimidines were developed as antibacterial drugs.[1–4] As bacteria have developed resistance to those drugs, new agents are needed for the treatment of bacterial infection. 6-Hydroxymethyl-7,8-dihydropterin pyrophosphokinase (HPPK) is another key enzyme in the folate pathway. It catalyzes the transfer of pyrophosphate from ATP to 6-hydroxymethyl-7,8-dihydropterin (HP) to form AMP and 6-hydroxymethyl-7,8-dihydropterin pyrophosphate (HPPP).[5–8]

Targeting HPPK, we previously synthesized a series of bisubstrate analogs by linking 6-hydroxymethylpterin (**1c**, Scheme 1) to adenosine through 2, 3, or 4 phosphate groups, and submicromolar affinity to the enzyme was obtained for the tetraphosphate inhibitor.[9] To improve the affinity and linker properties of such bisubstrate analogs, we have designed new compounds by replacing the pterin moiety (**1a**, Scheme 1) with 7,7-dimethyl-7,8-dihydropterin (**2a**, Scheme 1). Mimicking HP, **2a** has one more hydrogen bond donor than **1a** at position 8, which is important for binding to HPPK by forming a hydrogen bond with the carbonyl oxygen of a leucine residue of the protein.[10,11]

*Corresponding author. Tel: +1-301-846-5035; fax: +1-301-846-6073; jix@mail.nih.gov.

Stuart, Suckling, Wood, and colleagues[12–15] developed methods for the synthesis of both 6,7,7-trimethyl-7,8-dihydropterin (**2b**, Scheme 1) and 6-hydroxymethyl-7,7-dimethyl-7,8-dihydropterin (**2c**, Scheme 1), but **2c** was found to be degraded to 6-hydroxy-7,7-dimethyl-7,8-dihydropterin (**2d**, Scheme 1) upon prolonged incubation with HPPK.[11] In addition, the synthesis of **2c** was troublesome and the yield was low. A number of modifications were made, but the yield was still low.[16] In contrast, **2b** was easier to synthesize and stable, and the yield was high.[12] In this study, we focused on the derivatization of **2b** at position 6.

The first reaction of **2b** derivatization was bromination. Previously, Stuart[13,14] prepared 6-bromomethyl-7,7-dimethyl-7,8-dihydropterin (**3a**, Scheme 2) and 6-dibromomethyl-7,7-dimethyl-7,8-dihydropterin (**3b**) with bromine in glacial acetic acid (Scheme 2, i and ii, respectively). Compound **3b** could be used to derive 6-aldehyde-7,7-dimethyl-7,8-dihydropterin (**4b**) at high temperature,[17–21] but we found that the yield of the reaction was poor (Scheme 2iii). In general, bromination of methyl aromatic compounds could give tribromomethyl aromatic compounds that could be hydrolyzed to carboxylic acid aromatic compounds with silver nitrate in aqueous ethanol or sulfuric acid,[22] but we found that although **2b** gave 6-tribromomethyl-7,7-dimethyl-7,8-dihydropterin **(3c**, Scheme 2iv), compound **3c** did not yield the suggested 6-carboxy-7,7-dimethyl-7,8-dihydropterin **(4c**, Scheme 2v). Instead, multiple products were found in the reaction mixture.

We attempted to make compound **2e** by reacting **3a** with amines (Scheme 2vi). The desired products could be detected by LC-MS, but the products decomposed to a yellow fluorescent compound that was identified as **4b** (Scheme 2vii). To test whether we could derive **2e** from **4b**, we needed a better method for the synthesis of **4b**. Using direct oxidation of methyl group by SeO_2 as O'Neal and Jacobi reported previously,[24] we derived **4b** from **2b** and the yield of **4b** was 90% (Scheme 3).

Our method for the synthesis of **4b**: A solution of 2**b** (207 mg, 1.0 mmol) in DMF (10 mL) and pyridine (105 uL, 1.30 mmol) was treated with SeO_2 (145 mg, 1.30 mmol) and stirred at room temperature for 5 h. The reaction was then heated to 80 °C for 15 min. The solvent was evaporated under high vacuum and the residue purified by flash chromatography (silica gel, methanol:dichloromerhane = 2:8) to give **4b** (199 mg, 0.9 mmol, 90%) as a yellowish powder. HRMS (ESI) calculated for $C_9H_{11}N_5O_2$ ($[M+H]^+$) 222.0981, found 222.0991; ^1H NMR (400 MHz, CD_3OD) δ 1.53 (6 H, s), 9.33 (1 H, s); UV (0.1%AcOH), 400 nm.

Indeed, with **4b**, compound **2e** could be made by reductive amination reacting with an amine (Scheme 2viii). Again, the desired products, which could be detected by LC-MS, decomposed to the starting material (Scheme 2vii). Taken together, compound **2e** could be made with either **3a** or **4b**, but the products would still hydrolyze to **4b** (Scheme 2). Previously, Suckling and co-workers[23,12] reported that **4b** was obtained by gently oxidizing **2c** or a pterin ether analog (**5a**) (Scheme 2, ix and x, respectively).

Compound **2e** was not stable. We thought that the methylene group conjugating C=N could be a problem, and that an amide could improve the stability. Therefore, our second derivative of **2b** contains a carboxyl group linked to the pterin moiety. For the synthesis of carboxylic acid substituted aromatic compounds from methyl or aldehyde substituted aromatic compounds, various methods with the use of oxidation reagents can be found in the literature.[22] However, we found that these methods were not applicable for the synthesis of **2b** derivatives. When **2b** was mixed with the oxidation reagents, the characterized fluorescence of the compound disappeared.

For the synthesis of **3a**, a variety of bromination reagents were tested, including bromine, triphenylphosphine dibromide, N-bromosuccinimide, and phosphorus tribromide. We also

tested a variety of solvents such as chloroform, carbon tetrachloride, ethanol, N,N-dimethylformamide, and tetrahydrofuran. Usually, this kind of reaction gives a mixture of mono, bi, and tribromomethyl compounds. However, when ethanol was used as the solvent and the reaction mixture was separated by silica gel chromatography, light-yellow plate-shaped crystals were found in the collecting tubes. These crystals had a blue fluorescence, different from **4b** (yellow fluorescent) and **3a** (green fluorescent). The crystals were collected and identified as a new compound, 6-carboxylic ethyl ester-7,7-dimethyl-7,8-dihydropterin (**6a**, Scheme 4). We report here that **6a** was produced by heating **2b** with bromine in an ethanol solution (Scheme 4).

To generate **6a**, **2b** (0.5 g, 2.4 mmol) was dissolved in 80 ml ethanol in a heavy wall pressure vessel, bromine (0.43 ml 8.4 mmol) was added dropwise to the solution, and the pressure vessel was sealed with a Teflon bushing. The solution was heated overnight at 120 °C. The ethanol was evaporated and the residue was purified by a Teledyne column chromatography system. The desired compound **6a** (35%) was obtained as a yellow solid. *N*-bromosuccinimide (NBS) is one of the best bromination reagents. Using NBS instead of bromine in this procedure, however, the yield of **6a** was similar. HRMS (ESI) calculated for $C_{11}H_{15}N_5O_3$ ($[M+H]^+$) 266.1248, found 266.1270; ^{1}H NMR (400 MHz, CD_3OD) δ 1.52 (6 H, s), 1.32 (3 H, t), 4.23 (2 H, q); UV (0.1% AcOH), 380nm.

Goswami and coworkers[25] reported the side chain bromination of methyl heteroaromatic and aromatic compounds by solid phase NBS reaction under microwave to produce dibromo and carbaldehyde heterocyclic compounds. We tested the reaction with microwave but in solution. Compound **2b** (0.5 g, 2.4 mmol) and NBS (1.28 g, 7.2 mmol) were dissolved in 20 ml ethanol and the reaction mixture was heated in a Biotage microwave initiator for 5–25 min. The ethanol was evaporated and the residue was purified by the Teledyne column chromatography system. The desired compound **6a** (52%) was obtained as a yellow solid. We also added azobisisobutyronitrile (AIBN) in the reaction, but the yield (44%) was not improved. Bromine was also tested in the microwave reaction system. Due to a pressure-related issue, we had to decrease the reaction temperature and extend the reaction time; however, the yield (14%) was poor.

Overall, the microwave-assisted reaction had higher yield than conventionally heated reaction, but the microwave gave one byproduct which was identified to be **2d**: HRMS (ESI) calculated for $C_8H_{11}N_5O_2$ ($[M+H]^+$) 210.0986, found 210.0988; ^{1}H NMR (400 MHz, CD_3OD) δ 1.39 (s). The removal of the byproduct, however, was difficult in the reaction workup. In this reaction, NBS and bromine played the same role. Without the microwave, we prefer the use of bromine because the reaction was cleaner.

In summary, we synthesized a novel intermediate, 6-carboxylic acid ethyl ester-7,7-dimethyl-7,8-dihydropterin (**6a**, Scheme 4), and found a new way to derive 6-aldehyde-7,7-dimethyl-7,8-dihydropterin (**4b**, Scheme 3). With these two compounds, it is easy to extend the side chain at position 6 of 7,7-dimethyl-7,8-dihydropterin (**2a**, Scheme 1). Such derivatives of these two compounds can be used as antifolate agents (antibacterials, antimalarials, and anticancer drugs),[3] as nitric oxide synthase activators for the treatment of cardiovascular diseases,[26,27] and as pteridine reductase inhibitors targeting African sleeping sickness and the Leishmaniases.[28] The method can also be applied to other systems from methyl heteroaromatic and aromatic compounds to carboxylic ester heteroaromatic and aromatic compounds.

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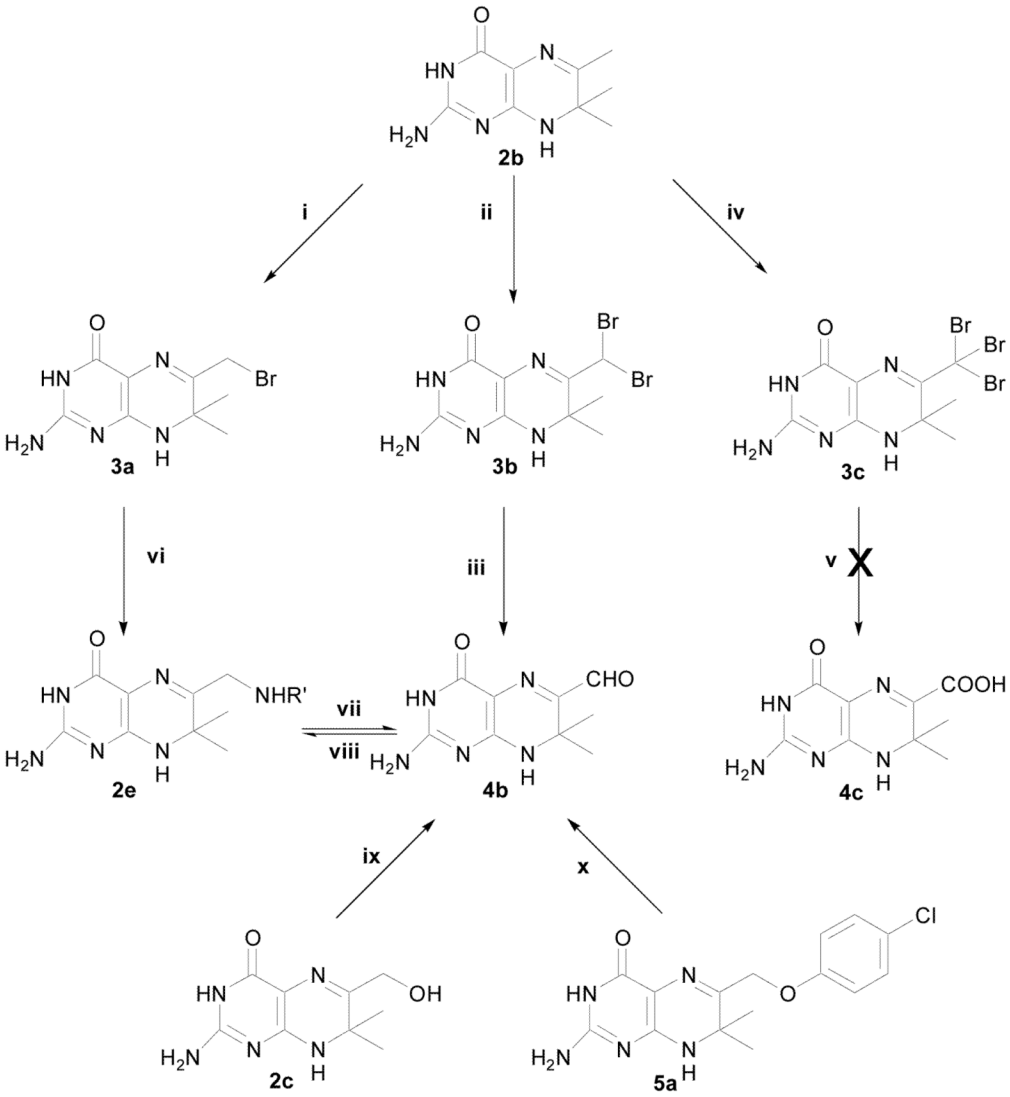
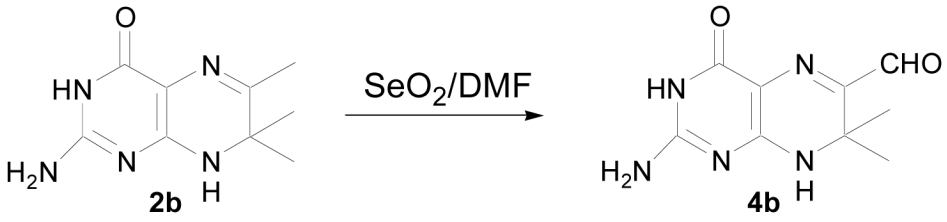
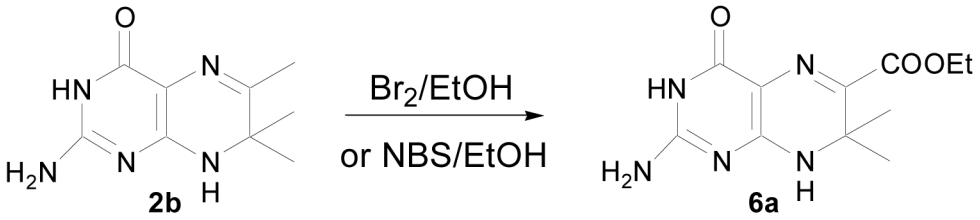
Scheme 1.
Pterin compounds: (**a**) R is absent, (**b**) R=CH_3, (**c**) R=CH_2OH, (**d**) R=OH, (**e**) R=CH_2NHR'.



Scheme 2.
Derivatization of compound **2b**: (**i**, **ii**) as reported,[13,14] (**iii**–**viii**) this work, (**ix**, **x**) as reported.[23,12]



Scheme 3.
New method of deriving 6-aldehyde-7,7-dimethyl-7,8-dihydropterin (**4b**) from 6,7,7-trimethyl-7,8-dihydropterin (**2b**).



Scheme 4.
Preparation of 6-carboxylic ethyl ester-7,7-dimethyl-7,8-dihydropterin (**6a**).