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A new route for total synthesis of (±) dephospho Form B of molybdenum cofactor by direct one step thiophene annulation from suitable pterin alkynes

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Dedicated to Professor Edward C. Taylor of Princeton University, USA on his 90th birthday for his well-known contribution in heterocyclic, medicinal, and thallium chemistry

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ABSTRACT

A new convenient total synthesis of (±) dephospho Form B of molybdenum cofactor has been described. The development of direct thiophene annulation onto pterin from a simple direct thiophene annulation at the alkyne moiety at 6-position of pterin is successfully achieved by using excess of sodium sulfide. This novel method conveniently led to the synthesis of a series of pterin fused α-substituted thiophenes (**9**–**11**, respectively).

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All oxomolybdoenzymes appear to require a common cofactor (molybdenum cofactor) as their catalytic center.[1,2] Molybdenum cofactor (Moco) is essential for the function of the human enzymes for example sulfite oxidase, xanthine dehydrogenase/oxidase, and aldehyde oxidase. The presence of pterin moiety is the reason of blue fluorescence in the degradation products of the Moco. The common organic cofactor for the activity of the enzymes has been termed molybdopterin. The dephospho derivatives Form A, Form B, urothione, and camMPT (carboxamidomethyl derivative) have been isolated from molybdenum cofactor **1** (Moco) (Fig. 1).[1–5] A significant number of review articles have been published to discuss the biochemistry, spectroscopy, mechanism, and recent advances of the Moco containing enzymes and the Moco structure.[6–11] Several oxidative degradation products of molybdopterin have been synthesized.[12–23] The suggested ligation of sulfur ligands of molybdopterin is in accordance with the results of EXAFS (extended X-ray absorption fine structure) studies.[5a] Sulfur functionalities are attached with the α and β unsaturated carbons to the pterin ring in camMPT, Form B, and urothione and the formation of these compounds is consistent with the decomposition of proposed 1,2-ene-dithiolate structure of Moco. The decomposition of Moco-containing molybdoenzymes was carried out in the absence of iodine and a degradation product dephospho Form B was obtained which contains only one of the two sulfur atoms present in molybdopterin itself, for which the structure (thieno[3,2-*g*]pterin) is shown as compound **12**.[23] In 1984, Form B structure was proposed by Johnson and Rajagopalan and co-workers based upon proton NMR and mass spectral analysis.[24]

Thus Form B and urothione are the two thiophene fused pterin metabolites which have been isolated from Moco from human urine. Taylor et al. have reported[13–15,22,24,26] the chemical synthesis of the dephospho Form A (asymmetric and racemic synthesis), Form B, deoxyurothione, and urothione. We describe in this Letter a novel, concise, and convenient total synthesis of (±) dephospho Form B (**12**) (from the pivaloyl and silyl protected thienopterin **11**) by a simple elegant direct thiophene annulation method onto a pre-formed suitable pterin alkyne developed by us. We also report here that this method conveniently led to the synthesis of a series of pterin fused α-substituted thiophenes (**9**, **10,** and **11** respectively).

The key to success of our direct thiophene annulation onto pterin having suitable alkyne moiety at 6-position was the anticipation

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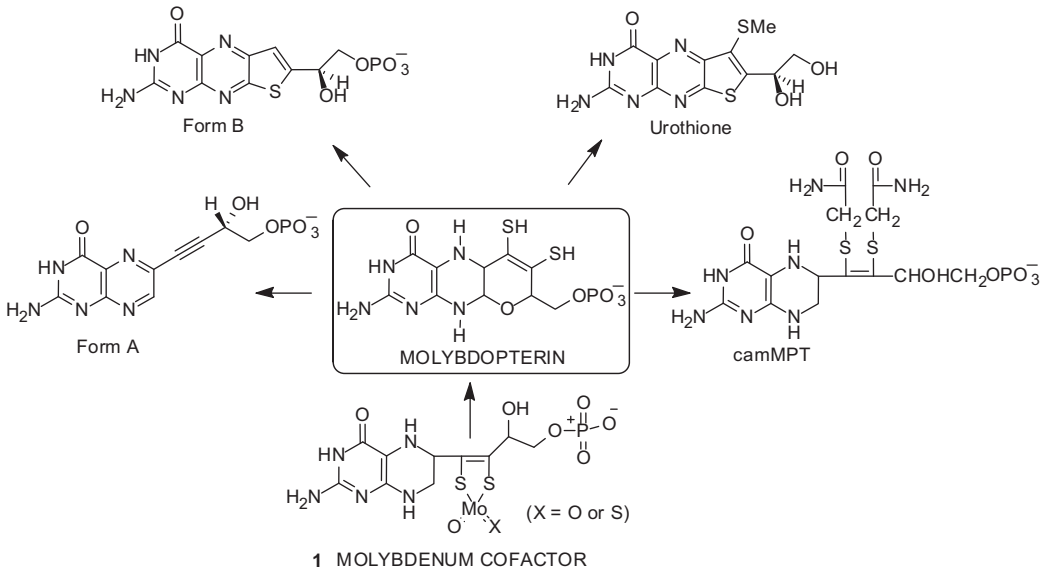


Figure 1. Structures of the Moco, MPT, and four degradation compounds of MPT.

that sulfide ion (S^{2-}) is nucleophilic enough to attack the acetylenic carbon attached with electron withdrawing pterin system (Michael addition) and then attack further in an intramolecular way the electron deficient imine carbon at 7-position of pterin for cyclization which really happened to our satisfaction. Thus one step direct thiophene annulation onto pterin from suitable pterin alkynes was successfully achieved simply in one step by sulfide ion (a very strong nucleophile, nucleophilicity order is 3.3).

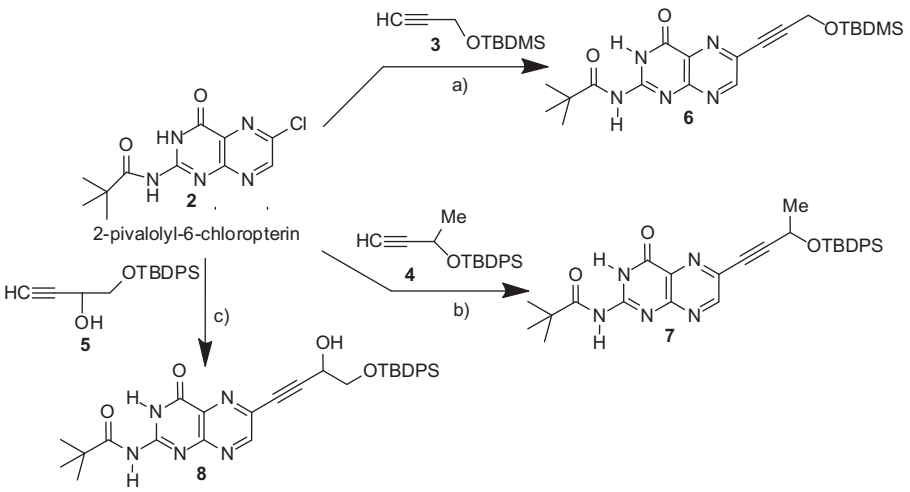
To follow the above synthetic strategy, we wanted to synthesize the suitable alkyne substituted pterins to reach the target thienopterins. While a broad range of *N*-pivaloylated-C(6)-alkyne substituted pterins (PPt–C≡C–CHRR$_1$ where PPt is 2-*N*-pivaloylpterin)[15,17] can be prepared following the procedure reported. [15,24,25]

Thus the alkyne functionality in compounds **6** and **8** were obtained by a palladium catalyzed coupling reaction of the corresponding terminal alkynes with C(6)-chloro-*N*-pivaloylpterin according to the procedure reported by Taylor et al.[15b,26a] (Scheme 1).

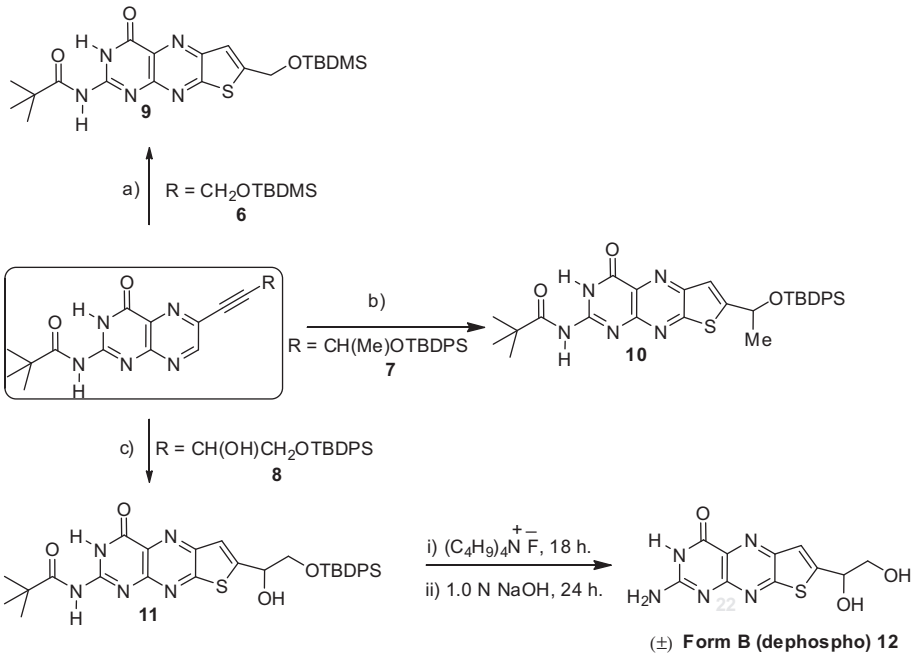
Now we describe the synthesis of thienopterins and hence Form B (dephospho). Taylor and Sabb[13] have reported a method for generating the highly functionalized thieno[2,3-b]pyrazine which was later applied to the synthesis of thieno[3,2-g]pterin. In our case, we used the pterin alkynes (**6**, **7** and **8**) for the thiophene annulation onto pterin by treating simply with sodium sulfide leading to the synthesis of thiophene fused pterins **9**, **10,** and **11** respectively of which **11** was converted into target Form B (**12**). The details of the synthesis are delineated in Scheme 2 (see also Supplementary data).

Thus thienopterins **9** and **10** have been readily accessible from alkynylpterins **6** and **7**, respectively by the reaction with excess sodium sulfide in THF. Similarly, treatment of **8** with sodium sulfide in THF gave the pivaloyl derivative **11** in 69% yield. The progress of the thiophene annulation reaction was monitored by TLC which showed the appearance of a more polar spot compared to the starting material and within a few hours it showed disappearance of the starting material.

The material **11** as isolated was shown by mass spectroscopy to have a molecular ion at *m*/*e* 602 (MH^+) and its ^{1}H NMR spectrum was consistent with the expected structure **11**. We have thus



Scheme 1. Synthesis of alkyne substituted pterins **6**–**8**. Reagents and conditions: (a) $Pd(OAc)_2$, CuI, PPh_3, Et_3N, MeCN, 60 °C, 4 h, 76%; (b) $Pd(PPh_3)_4$, CuI, Et_3N, MeCN, 60 °C, 3 h, 72%; (c) $Pd(OAc)_2$, (*o*-tolyl)$_3$P, CuI, MeCN, 100 °C, 3.5 h, 62%.



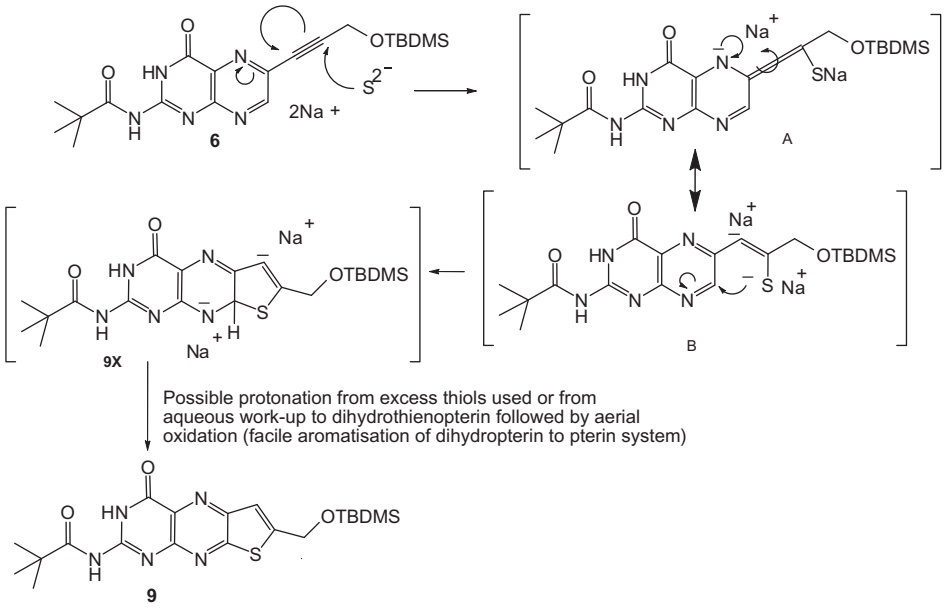
Scheme 2. Synthesis of thienopterins **9**–**11** and dephospho (±) Form B (**12**). Reagents and conditions: (a) excess Na_2S, dry THF, rt, 1 h, 63%; (b) excess Na_2S, dry THF, rt, 4 h, 65%; (c) excess Na_2S, dry THF, rt, 4 h, 47%.

successfully and conclusively demonstrated that the thieno[3,2-*g*]pterin ring system can be formed from alkynylpterin. The remaining part of the synthesis of Form B was straightforward. The thienopterin **11** was treated with tetrabutylammonium fluoride to cleave the TBDPS group and then hydrolysis with 1.0 N NaOH in aqueous solution at room temperature gently removed the pivaloyl group to give the desired (±) Form B (dephospho) **12** the structure of which was confirmed by comparison of spectroscopic data of the reported (±) Form B (dephospho) **12** isolated from human urine.[24c,23d]

The thiophene annulation is confirmed by the NMR spectral data which show the absence of pterin H-7 peak of the pyrazine ring of **6**, **7,** and **8** at δ 8.63, 8.80, and 8.77, respectively and the appearance of thiophene H-3′ of **9**, **10,** and **11** at δ 7.34, 7.37, and 7.38, respectively. The NMR of dephospho Form B shows H-3′ of the thiophene ring at δ 7.37 (s) (Supplementary data).

The possible mechanism for thiophene annulation is explained in Scheme 3. The first Michael attack of nucleophile S^{2-} to the alkyne possibly results in an allene (**A**) which is resonance stabilized (**B**) which follows an intramolecular attack of thiolate ion (**B**) to give rise to thiophene fused dihydropterin system (**9X**) which on aerial oxidation for facile aromatization affords the target thienopterin **9**.

Thus, we have successfully utilized the advantages of conjugated acetylene moiety at the 6-position of the pterin system; (1) easy conversion of the corresponding pterin anion (negative charge



Scheme 3. Plausible mechanism for the formation of thienopterin **9**.

at N5) formed by nucleophilic attack by sulfide to the triple bond or subsequently produced dihydropterin to aromatized pterin by oxidation[27] and (2) the superior nucleophilicity of naked sulfide ion to result in direct thiophene annulation onto pterin.

Thus our strategy provides the first one step synthesis of thienopterin **9**, from pterin-substituted acetylene **6** (Scheme 2). Previously Joule et al. reported[28] thiophene annulation on quinoxaline from dibromo derivative of 2-alkynylquinoxalines with dipotassium trithiocarbonate.

We thus report here a convenient and concise total synthesis of thienopterins and (±) Form B (dephospho) **12**. The synthesis of thienopterins and ultimately Form B has evolved from a simple novel method of efficient formation of dithiolenes and thienopterin from the suitable acetylene moiety attached with pterin ring system. The specific thiophene annulation onto pterins is thus demonstrated by the direct synthesis of thienopterins **9**–**11** in good yield.

Our method of synthesis is significant to establish a synthetic correlation between Form A derivative (pterin alkyne with a suitable substituent at the alkyne moiety) and Form B, which are derived as decomposition products from Moco.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.02.090.

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