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PREPARATION OF 2-CARBOXY-3,4-SUBSTITUTED PYRROLE HAPTENS AND SYNTHESIS OF PORPHOBILINOGEN

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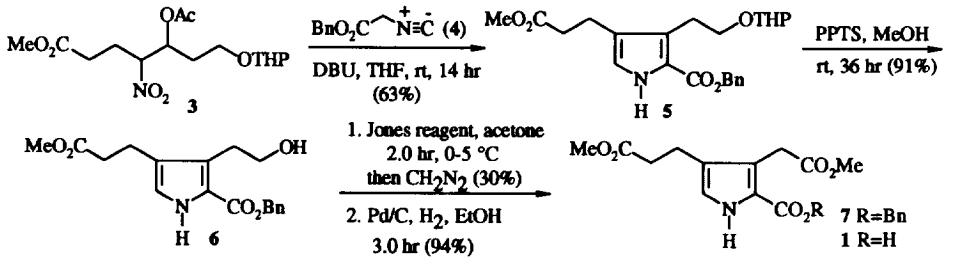
Summary: *2-Carboxy-3,4-substituted pyrrole **1**, the porphobilinogen (PBG, **2**) like hapten was prepared via condensation of α-acetoxynitro compound (**3**) with benzyl isocyanoacetate (**4**) and further functional group transformations in good yields. Also, the hapten **1** was converted to PBG (**2**).*

Lead (Pb) poisoning continues to be a significant and devastating environmental disease in the pediatric population. The adverse effects of exposure to lead are seen in the development of the brain and the nervous system in young children and also, anemia, lethargy or peripheral neuropathy are commonly evident with blood lead concentration at 70 μg/dl (3.38 mmol/L).[1] Heavy metals such as lead are know to inhibit the activity of aminolevulinic acid dehydratase (ALAD) enzyme which catalyses the in vivo dimerization process of 5-aminolevulinic acid to form a molecule of porphobilinogen (PBG, **2**), the key building block in the biosynthesis of "pigments of life" such as porphyrins, chlorophylls, corrins and vitamin B_{12}.[2,3] The determination of the ALAD enzyme activity by quantifying the condensation products (PBG, **2**) has been shown to be a useful marker in the measurement of lead.[4] Recently, for our program on the development of lead assay, we needed a PBG (**2**) like haptens for the preparation of various immunoreagents. In this paper, we describe a convenient preparation of 2-carboxy-3,4-substituted pyrrole **1**, which is an important hapten for the development of lead assay, and its conversion to porphobilinogen (**2**)[5]

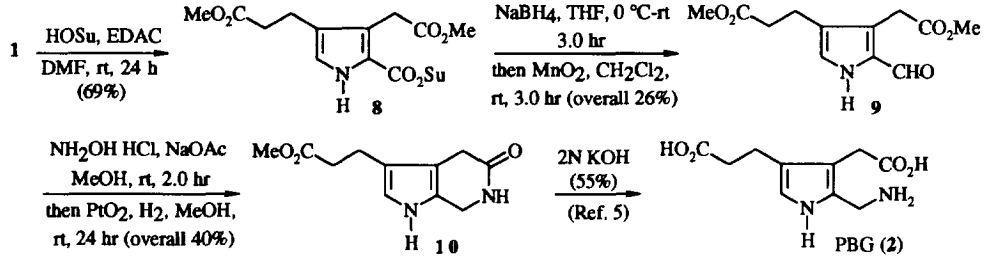
Scheme 1



The strategy (scheme 1) for the construction of 2-carboxy-3,4-substituted pyrrole **1** hapten, involves the condensation of α-acetoxynitro compound **3** with benzyl isocyanoacetate (**4**), and further functional group transformations. Accordingly, α-acetoxynitro compound **3**[5] was condensed with benzyl isocyanoacetate (**4**) in THF using DBU (1.4 equiv.) at rt for 14 hr, to afford 2-benzyloxycarbonyl-3,4-substituted pyrrole **5** in 63% yield after silica gel column chromatography (35% EtOAc in *n*-hexane) purification.[6,7] The tetrahydropyranyl ether in **5** was cleaved using pyridinium *p*-toluenesulfonate (PPTS, 1.05 equiv.) in methanol at rt for 36 hr to afford the alcohol **6** in excellent yield (91%). The hydroxy functionality in **6** was then converted to methyl ester **7**, first by oxidation with Jones reagent (2.02 equiv.) in acetone at 0-5 °C for 2 hr followed by esterification of

the resulting crude acid with diazomethane in ether-ethyl acetate (1:1) solvent at 0 °C and purification by silica gel column chromatography (30% EtOAc in *n*-hexane), in 30-32% yield.[8] Hydrogenation of the benzyl ester **7** using 10% Pd/C in ethanol for 3.0 hr afforded the 2-carboxy-3,4-substituted pyrrole **1** in 94% yield.

Scheme 2



The hapten **1** was then converted (scheme 2) to porphobilinogen (**2**). Accordingly, **1** was treated with N-hydroxy succinimide (HOSu, 2.0 equiv.) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC, 3.0 equiv.) in DMF at rt for 24 hr to produce N-succinimido ester **8** in 69% yield.[9] The active ester **8** was then reduced with sodium borohydride (2.5 equiv.) in THF at 0 °C to rt for 3 hr to give the corresponding unstable alcohol which was immediately oxidized with manganese (IV) oxide (MnO_2, <5 micron, activated, ~85%, 3.0 equiv.) in $CHCl_3$ at rt for 3 hr to afford the aldehyde **9** in 26% overall yield.[10] The aldehyde **9** was treated with hydroxylamine hydrochloride and sodium acetate in methanol for 2.0 hr at rt and the resulting oxime was subjected for hydrogenation using Adam's catalyst (PtO_2) in methanol for 24 hr. Purification of the crude product by silica gel preparative TLC (6% methanol in $CHCl_3$) afforded the methyl ester of PBG lactam **10** in 40% yield. The lactam **10** was previously converted to PBG (**2**) by alkaline hydrolysis in 55% yield.[5] In summary, 2-carboxy-3,4-substituted pyrrole **1**, an important hapten for the preparation of immunoreagents for lead assay was prepared from α-acetoxynitro compound **3** and benzyl isocyanoacetate (**4**), and also a versatile synthesis of porphobilinogen (**2**) was accomplished. The application of acid **1** as well as other haptens (ex. **6, 9, 10**) for the preparation of immunoreagents is in progress and will be described in due course.

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