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DIAZOCYCLOHEXADIENONES AS PHOTOAFFINITY LIGANDS: SYNTHESES OF TRIOXABICYCLOOCTANE PROBES FOR THE CONVULSANT BINDING SITE OF THE $GABA_A$ RECEPTOR

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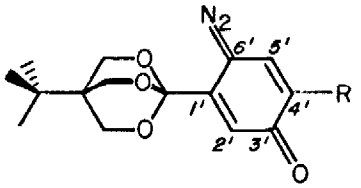
Summary: Photoaffinity ligand **2** combines the bromodiazocyclohexadienonyl and t-butyltrioxabicyclo-[2,2,2]octane moieties to achieve suitable photochemical properties and affinity for the convulsant binding site of the $GABA_A$ receptor.

Characterization of the convulsant binding site is an important step in further understanding the γ-aminobutyric acid (GABA)-modulated chloride channel. The $GABA_A$ receptor consists of an α subunit with multiple subtypes and a β subunit[1]. A reactive probe is needed to determine which subunit(s) of the receptor complex is(are) irreversibly labeled and thus comprise(s) the convulsant binding site. This binding site on cerebral membranes has been characterized using three radioligands, i.e., dihydropicrotoxinin[2] and the bicyclic convulsants t-butylbicyclophosphorothionate[3] (TBPS) and 4-t-butyl-1-phenyl-2,6,7-trioxabicyclo[2.2.2]octane (also known as t-butylbicycloorthobenzoate or TBOB)[4,5].To our knowledge, the para-azido derivative of TBOB is the only potential photoaffinity probe described for the convulsant binding site[4]. We report here the syntheses of two diazocyclohexadienonyl t-butyltrioxabicyclooctanes, **1** and **2**.



1 R = H
2 R = Br

4-Diazocyclohexa-2,5-dienones constitute a new class of photoactivatable reagents used recently to irreversibly label the catalytic site of acetylcholinesterase[6]. This photosensitive function is preferred for the high reactivity of the corresponding photogenerated carbene which for instance inserts in C-H bonds[7] and even reacts with molecular nitrogen[8]. The molecular characterization of a binding site which may involve several overlapping polypeptide chains is best achieved by the use of such highly reactive probes.

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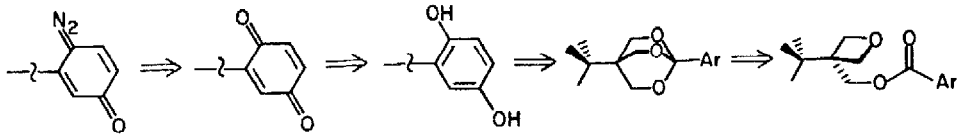
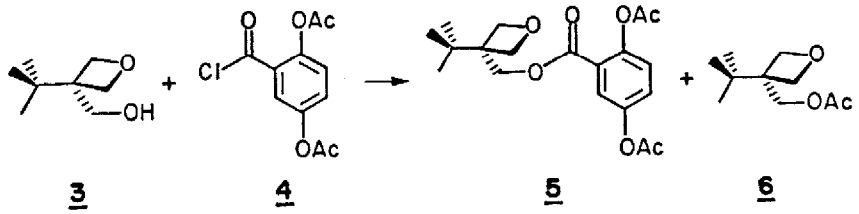
The general synthetic pathway is based on the retro-synthetic scheme shown below.



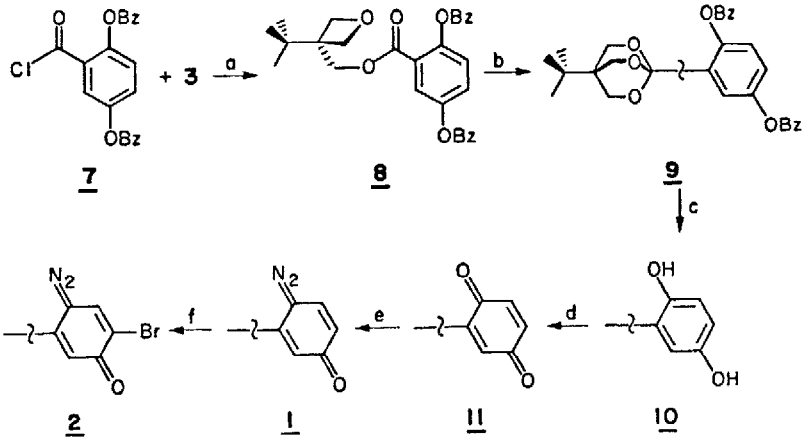
Syntheses of diazocyclohexadienones are generally achieved either by diazotization of the corresponding aminophenol or by basic treatment of the tosylhydrazone derived from the corresponding para-quinone[9], which is readily accesible from the para-hydroquinone. The latter route was chosen because of the acid sensitivity of the bicyclic orthobenzoates. The para-hydroquinone moiety is introduced into the bicyclic ring system via the oxetane ester involving BF_3-etherate catalyzed cyclization[4,10].

Gentisic acid was used as the precursor of the quinonic moiety following protection of the phenolic groups. Condensation of the oxetane alcohol **3**[4] with the acid chloride of gentisic acid diacetate **4**[11] in the presence of pyridine yielded mainly polycondensed compounds. As an alternative, the lithium alkoxide of **3** gave, in addition to the expected ester **5**, the oxetane acetate **6**.



3 **4** **5** **6**

The preferred dibenzyl derivative **7**[12] led to the successful synthesis of **1** and **2**. Oxidation of the bicy-



7 **8** **9** **10** **11** **1** **2**

a: (65% yield) pyridine, THF; **b**: (70% yield) $BF_3.Et_2O$, CH_2Cl_2; **c**: (75% yield) H_2,Pd, EtOAc;
d: (95% yield) Ag_2CO_3 on celite, C_6H_6; **e**: (30% yield) 1.$TsNHNH_2$, CH_2Cl_2, 2. Al_2O_3;
f: (90% yield) 1. Br_2,CH_2Cl_2, 2.Et_2NH.

clic hydroquinone **10** with Ag_2CO_3 on Celite[13] gave quinone **11** almost quantitatively. A diazotization step using tosylhydrazine[9] followed by chromatography on alumina led suprisingly to the formation of only one isomer, the 6'-diazocyclohexa-1',4'-dienone **1**[14]; no 3'-diazo isomer was detected. Bromination of **1** followed by basic dehydrobromination gave exclusively **2**[15] in a regioselective reaction involving addition of bromine on the less hindered double bond followed by an E2 elimination involving the more acidic hydrogen in the 4'-position.

Both **1** and **2** display the typical UV spectra of 4-diazocyclohexa-2,5-dienone derivatives (Figure 1). In the absence of light, both are stable in aqueous buffer at pH 7.4. Reaction on the chromophore is easily monitored by UV spectroscopy and on the bicyclic system by NMR spectroscopy (disappearance of the 4.12 ppm signal). Irradiation of an aqueous solution of either chemical led to complete disappearance of the higher absorption band as shown in Figure 1. The occurrence of isosbestic points is in agreement with nearly quantitative formation of the corresponding hydroquinone[16]. In addition, the absorption spectra of both **1** and **2** are compatible with a tryptophan-mediated energy transfer photodecomposition[17].

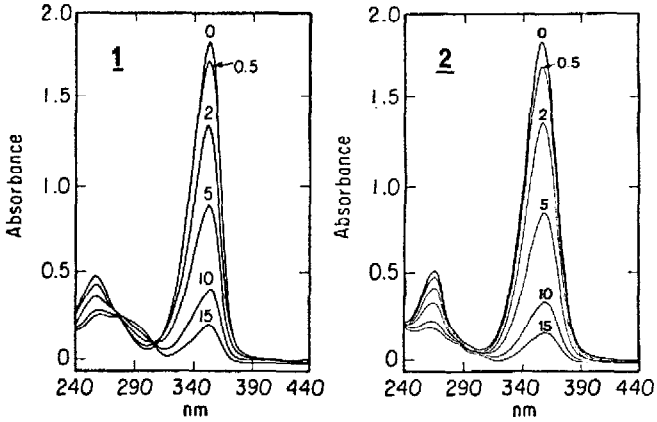
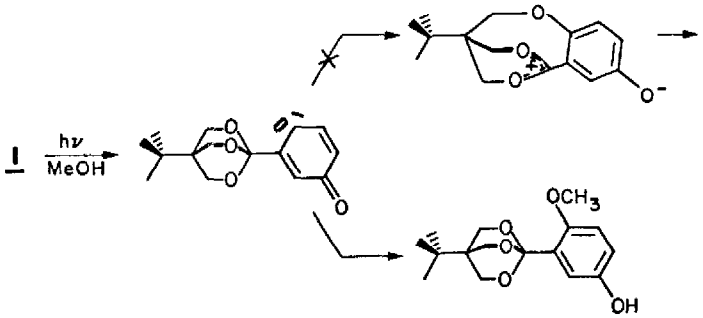


Figure 1: Photodecomposition at 350nm of **1** and **2** in water (times in min.)

Ideal photoaffinity probes should not rearrange photochemically to produce less reactive intermediates. A possible intramolecular photorearrangement involving reaction of the trioxabicyclic moiety on the carbene does not occur during photolysis of **1** in methanol but instead the rapid reaction of methanol on the photogenerated carbene yields anisole derivative **12**.



12

Diazocyclohexadienones **1** and **2** inhibit the binding of [^{3}H] TBOB to bovine cerebral cortical membranes with IC_{50S} of 11 and 1 μM, respectively. This eleven-fold increase in binding affinity due to bromine in the 4'-position is consistent with the described increase in potency observed with halogenated derivatives of bicyclic orthobenzoates[4]. In the absence of light, the inhibition of [^{3}H] TBOB binding by **1** and **2** is reversible. Preliminary photoinactivation experiments show that **2** irreversibly blocks the [^{3}H] TBOB binding site, e.g., irradiation of the membrane preparation in the presence of 20 μM **2** at 350nm leads to over 35% irreversible inhibition and this inactivation is partially protectable by unlabeled TBOB.

This is the first report on the successful preparation of an irreversible photoaffinity ligand to probe the convulsant site of the $GABA_A$ receptor.

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14 Compound **1**: Yellow solid, mp (dec.) 188°C, NMR 300 MHz (CDCl3) δ 0.91 (9H, s., $(CH_3)_3C$) ; 4.12 (6H, s., $(CH_2O)_3$) ; 6.39 (1H, d.d., $H_{4'}$, $J_{H4'-H5'}$ = 10Hz, $J_{H4'-H2'}$ = 2Hz) ; 6.91 (1H, d., $H_{2'}$) ; 7.34 (1H, d., $H_{5'}$).
15 Compound **2**: Yellow solid, mp (dec.) 90°C, NMR 300 MHz (CDCl3) δ 0.91 (9H, s., $(CH_3)_3C$) ; 4.12 (6H, s., $(CH_2O)_3$) ; 6.76 (1H , s., $H_{2'}$) ; 7.76 (1H , s., $H_{5'}$).
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