Aryldiazirine-Modified Pyroglutamates: Photoaffinity Labels for Glutamate

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Abstract: The synthesis of an aryldiazirine-modified pyroglutamate is reported for potential application as a photoaffinity label at glutamate receptors. Detailed ^{13}C NMR and ^{19}F NMR spectroscopic characterisation data for these trifluoromethyl-substituted aryldiazirines has been determined and shown to be useful for tracking this group during synthesis.

Key words: glutamate, photoaffinity, diazirines

Glutamate is a key player in diverse biological processes, including amino acid metabolism (e.g., where it is an important couple with α-ketoglutarate in deamination processes) and the mammalian central nervous system (where it acts on ionotropic and metabotropic receptors).[1] As a consequence, the control of inter- and intracellular glutamate concentration is of paramount importance; membrane-bound glutamate transporters play a key role in this regard. Much effort has therefore been invested in the identification and structural elucidation of various glutamate receptors, and although many of these are membrane bound, structural details are beginning to emerge.[2,3] Caged glutamate has also been developed for the controlled release of glutamate into biological systems,[4] and photoaffinity labelling has been used for the identification of amino acid residues at enzyme active sites or receptor binding sites.[5–7] Of the possible photoprecursors, diazirines exhibit excellent photolytic properties,[8,9] but are less readily synthetically accessible than the more commonly used azides or nitroaryl derivatives. We have previously demonstrated the utility of aryldiazirines as photaffinity labels readily activated by laser irradiation,[10] and have become interested in the application of this approach for the determination of binding site information for excitatory amino acid (EAA) receptors. Azido-derived photoaffinity labels have recently been investigated for the NMDA receptor[11,12] and KA receptors,[13–17] and there has been a recent report of a selective mGluR1 radioligand,[18] but there are no reports of the incorporation of diazirine systems into EAA receptor agonists. Reports by Hatanaka[19] and Brunner[20] indicated that 3-methoxyphenyldiazirines **1a** could be thallated and further reacted with a variety of electrophiles under mild conditions, thereby permitting introduction of an aryldiazirine unit intact into a substrate and avoiding a lengthy linear synthesis. However, the obvious drawbacks with the use of thallium prompted the development of a Friedel–Crafts alkylation strategy for conversion of 3-methoxyphenyldiazirine **1a** to 4-aldehyde **1b** or 4-hydroxymethyl derivative **1c** (Scheme 1).[21] The utility of this approach has been demonstrated by the introduction of the 3-methoxyphenyldiazirine unit **1a** into a range of biological probes.[22–31] Of interest is the rapid photolysis of the diazirine function in these compounds (typically $t_{1/2}$ for photolysis with a 15 W UV lamp is 1.7 min).[26] Although the facile preparation of bromide **1d** has also been reported,[21] its application as a diazirinyl carrier has not been widely applied, and so far has only been used for the preparation of a photoactivatable phenylalanine analogue.[32] We report here that bromide **1d** is applicable for the incorporation of aryldiazirines into sterically hindered pyroglutamate systems of relevance to glutamate receptor structural investigations.

3-Methoxyphenyldiazirine **1a** was readily prepared in high overall yield in multigram quantities from 3-bromoanisole in 5 steps using our previously published protocol (Scheme 1);[33] this route differs slightly from an alternative literature method,[21] in that commercially available methyl trifluoroacetate is used for the initial acylation step and iodine/triethylamine for the final oxidation. Friedel–Crafts alkylation followed by aqueous work-up yielded aldehyde **1b** (62%), along with a lesser amount of isomer **2b** (16%). The stereochemical assignment for each of these was readily confirmed by NOESY analysis, which exhibited the indicated correlations (Figure 1), confirming the earlier assignment.[21] Conversion of aldehyde **1b** to bromide **1d** via alcohol **1c** was readily achieved using the literature procedure.[21] Of interest is that electrophilic bromination of anisole **1a** was also found to be readily possible; thus, bromination (Br_2, $TiCl_4$, CH_2Cl_2) of **1a** gave a separable 3.2:1 mixture of isomeric bromides **1e** and **2e** in an overall yield of 55%; the stereochemistry of **2e** was again determined by NOESY analysis (Figure 1). Noteworthy is the difficulty of demonstrating the presence of the diazirine unit in these compounds, since its UV chromophore is very weak (typically, ε is less than 1000), and mass spectroscopic analysis is unreliable due to the facile extrusion of N_2; we found that reliable indicators for the presence of the diazirine subunit were the ^{13}C (ca. 122 ppm) and ^{19}F (ca. –65.0 ppm) chemical shift and $^2J_{C-F}$ (ca. 274 Hz) coupling constant values for the adjacent CF_3 unit, which could be readily observed (Table 1). Mercuration [$Hg(O_2CCF_3)_2$ in HO_2CCF_3 at r.t. for 30 h] of substrate **1a** gave exclusively derivative **2f**, presumably as a result of chelation control, but direct plumbation of the aromatic ring of **1a**

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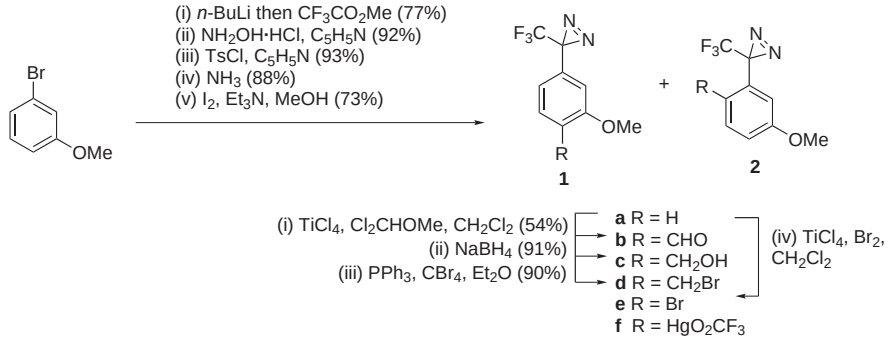


Scheme 1

or attempted mercury–lead exchange of **1f** gave an intractable mixture. The assignment of the regiochemistry of **1f** is based on a report that substitution of an aromatic ring with mercury does not change chemical shift values of the remaining protons around the ring;[34] the position of substitution is therefore apparent by identification of the missing proton relative to the starting material.

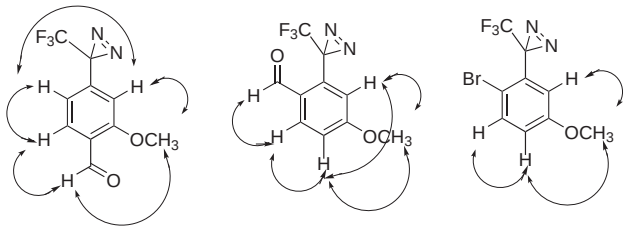
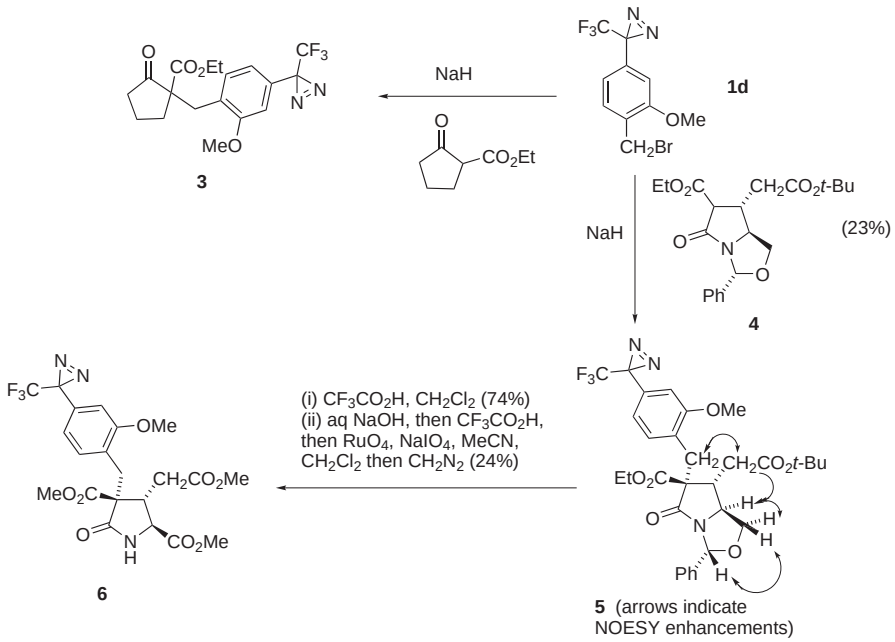


Figure 1

The utility of these substrates for inclusion in sterically hindered pyroglutamate-derived substrates was demonstrated by the successful alkylation of ethyl 2-oxocyclopentanecarboxylate with bromide **1d** to give cyclopentanone **3** in a yield of 54% using NaH as the base (Scheme 2). When this procedure was applied for the synthesis of lactam **4**, prepared as a key intermediate in our recently published synthesis kainoid analogues,[35] with aryl bromide **1d**, adduct **5** was obtained as a single diastereomer in a yield of 23%.[36] The stereochemistry of this compound was confirmed by comparison of ^{1}H NMR chemical shift data for related compounds,[35] and by NOESY analysis, which clearly indicated the *exo*-orientation of the arylmethyl residue (see Scheme 2).[36] This product was elaborated to pyroglutamate **6** by acid-catalysed deprotection, ester hydrolysis, oxidation and esterification in good overall yield; in this compound, the ^{13}C NMR and ^{19}F NMR characterisation data (see Table 1) of the diazirine unit of **6** was consistent with that of its



Scheme 2

simpler precursors.[37] In contrast to the successful alkylations, however, mercury–lead exchange of **2f** with lead tetraacetate, followed by attempted Pinhey arylation of lactam **4**,[38] was not successful.

This route provides direct access to novel aryldiazirines, and we anticipate that this sequence will facilitate the development novel photoaffinity labels suitable for EAA receptors.

Table 1 ^{13}C NMR and ^{19}F NMR Chemical Shift and Coupling Constants for the CF_3 Unit in some Trifluoromethyldiazirines

Compound	^{13}C (ppm)	$J_{C–F}$ (Hz)	^{19}F (ppm)
1a	122.1	274.7	–66.94
1b	121.7	274.8	–64.68
2b	121.7	274.9	–68.70
1c	122.1	274.4	–65.16
1d	122.1	274.7	–64.99
1e	121.9	274.7	–
2e	121.7	275.6	–68.23
6	122.1	274.7	–64.93

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(36) **Data for Lactam 5.**
Obtained as a colourless oil (0.111 g, 23%); R_f = 0.20 [PE(40–60 °C)–EtOAc, 5:1]. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.30 (3 H, t, *J* = 7.0 Hz, CH_3CH_2), 1.47 [9 H, s, $C(CH_3)_3$], 2.66 (2 H, d, *J* = 7.6 Hz, CH_2CO_2t-Bu), 3.09–3.15 [1 H, m, H(6)], 3.23 (2 H, dd, *J* = 13.9 Hz, CH_2Ph), 3.33 (3 H, s, OCH_3), 3.60–3.66 [1 H, m, H(5)], 3.76–4.26 [2 H, m, H(4)], 4.30–4.34 (2 H, m, CH_3CH_2), 6.21 [1 H, s, H(2)], 6.39 (1 H, s, ArH *ortho* to OMe)], 6.47 (1 H, d, *J* = 8.0 Hz, ArH *para* to OMe), 7.23 (1 H, d, *J* = 8.0 Hz, ArH *meta* to OMe), 7.27–7.44 (5 H, m, PhH). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 14.1 (CH_3CH_2), 28.1 [$C(CH_3)_3$], 28.3 (CH_2Ph), 33.9 (CH_2CO_2t-Bu), 45.9 [C(6)], 54.7 (OCH_3), 61.9 [C(5)], 62.0 ($CO_2CH_2CH_3$), 63.9 [C(7)], 72.8 [C(4)], 86.9 [C(2)], 107.8 (ArC *ortho* to OMe), 118.3 (ArH *para* to OMe), 126.0, 126.5, 128.4, 128.7, 128.9 (all ArCH), 127.4 (q, *J* = 280.5 Hz, CF_3), 126.1, 129.1 (ArC), 132.6 [C(15)], 138.3 [(C(13)], 157.6 [C(11)], 170.6 (lactam C=O), 170.8 (ethyl ester C=O), 171.7 (*t*-Bu ester C=O). IR (thin film): ν_{max} = 2981 (s, aliphatic C–H), 1728 (br s, ester and lactam C=O), 1261 (s, C–O), 1039 (s, C–F) cm^{-1}.

(37) **Data for Lactam 6.**
Obtained as a colourless oil (0.0198 g, 24%). R_f = 0.23 [EtOAc–PE (40–60 °C), 6:5]. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.73–2.85 (2 H, m, CH_2CO_2Me), 3.22 (2 H, dd, *J* = 14.7 Hz, CH_2Ph), 3.52–3.62 [1 H, m, H(3)], 3.70, 3.74, 3.77, 3.79 (4 × 3 H, s, OCH_3), 3.69–3.76 [1 H, m, H(2)], 6.31 (1 H, s, NH), 6.55 (1 H, s, ArH *ortho* to OMe), 6.69 (1 H, d, *J* = 7.9 Hz, ArH *para* to OMe), 7.37 (1 H, d, *J* = 8.0 Hz, ArH *meta* to OMe). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 27.2 (CH_2Ph), 28.6 [C(3)], 32.3 (CH_2CO_2Me), 43.9 [C(2)], 52.0, 52.8, 53.0 (OCH_3), 55.2 ($ArOCH_3$), 58.6 [C(4)], 108.0 (ArC *ortho* to OMe), 118.7 (ArC *para* to OMe), 122.0 (q, *J* = 274.7 Hz, CF_3), 126.2 (ArC *ortho* to OMe), 129.3 (ArC), 131.7 (ArC), 157.6 [C(8)], 170.3, 170.7, 171.4 (C=O esters), 173.0 (C=O lactam). IR (thin film): ν_{max} = 3020, 2956 (m, C–H), 2401 (m, N=N), 1740 (s, C=O), 1577, 1515 (m, Ar C=C), 1216 (s, C–O), 1039 (s, C–F) cm^{-1}.

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