Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXIII, No. 7

SYNTHESIS OF NOVEL [^{14}C]-LABELLED PHENYLUREAS BEARING PHOTOACTIVE AZIDO AND DIAZIRINE GROUPS AS PHOTOAFFINITY LABELS FOR THE HERBICIDE BINDING SITE OF PHOTOSYSTEM TWO

Philip Jewess[1], Julian P. Whitelegge[2,3], Patrick Camilleri [1,4] and John R. Bowyer[2]

[1]Shell Research Ltd., Sittingbourne Research Centre, Sittingbourne, Kent ME9 8AG, U.K. [2]Department of Biochemistry, Royal Holloway, University of London, Egham Hill, Egham, Surrey TW20 0EX, U.K.
[3]Present address: Department of Biology, University of California, Los Angeles, 405 Hilgard Avenue, Los Angeles, California 90024-1606, U.S.A.
[4]Present address: SmithKline Beecham Research Ltd., The Frythe, Welwyn, Herts. AL6 9AR, U.K.

SUMMARY

We have synthesised two photoaffinity-labelling hydrophobic stilbene urea inhibitors of Photosystem 2 (PS2) bearing either an azido group (3-(3-((E)2-(4-azidophenyl)ethenyl)-phenyl)-1,1-dimethylurea, azido-PDU) or a diazirine group (3-(3-((E)2-(4-(3-(trifluoromethyl)-diazirin-3-yl)phenyl)ethenyl)phenyl)-1,1-dimethylurea, diazirinyl-PDU) as well as [^{14}C]-labelled forms of these compounds. The key step in both syntheses was the formation of the stilbene entity by a Waddsworth-Emmons reaction of either a trifluoroacetyl-protected aminobenzaldehyde or a 3-(trifluoromethyl)-diazirin-3-yl-substituted benzaldehyde with dimethyl(4-nitrophenylmethyl)-phosphonate which gave largely E stereochemistry. The latter compound was made from 4-bromobenzaldehyde protected as the 1,3 dioxolane. The substituted diazirine was then constructed via Grignard chemistry and an adaptation of a published procedure for the synthesis of diazirines. Reduction of the nitrostilbenes to the respective anilines and conversion to the isocyanates followed by reaction with di[^{14}C]methylamine then gave the required [^{14}C]-labelled phenylurea photoaffinity reagents. In studies not detailed here both reagents were found to be highly active inhibitors of photosynthetic electron transport before UV irradiation and to covalently label the herbicide-binding protein (D1) of Photosystem 2 after UV treatment. Diazirinyl-PDU was found to be considerably more sensitive to UV irradiation than azido-PDU.

Key words: phenylurea, herbicide, Photosystem 2, photoaffinity, azide, diazirine.

0362–4803/93/070655–15$13.50

Received 1 March, 1993
Revised 3 March, 1993

INTRODUCTION

It is now well established that many of the herbicides which block electron transport at Photosystem 2 (PS2) competitively displace plastoquinone at the so-called Q_B site on the D1 protein of PS2, thus blocking oxidation of the reduced primary quinone, Q_A, by Q_B [1-3]. Some of the PS2 herbicides also block cleavage of photo-damaged D1 polypeptide within PS2 thereby blocking recovery via replacement of the damaged D1 molecule [4]. There is considerable interest in determining the architecture of the Q_B site since it may facilitate the design of novel herbicides which interfere with the light-dependent D1 turnover cycle and/or inhibit electron transport.

The herbicide receptor protein in PS2 was first identified by photoaffinity labelling with [^{14}C]-azidoatrazine, a photoactivatable analogue of the triazine herbicide, atrazine [5]. The binding of the triazine herbicide, terbutryne, to photosynthetic reaction centres of the bacteria Rhodopseudomonas viridis and Rhodobacter sphaeroides has been studied by X-ray crystallography [6]. The herbicide-binding site in these bacterial photosynthetic reaction centres shows considerable homology with that in PS2 [7] and the crystallographic studies will undoubtedly assist in modelling triazine binding to D1. Phenylurea herbicides, however, do not bind to wild-type bacterial reaction centres, so that there has been less information available about their binding interactions with D1 in PS2.

QSAR studies of inhibitors binding to PS2 have indicated the presence of a hydrophobic cleft adjacent to the herbicide binding site which could be used to increase binding potency of inhibitors via attachment of a suitably located hydrophobic moiety [8-11]. The isoprenoid side chain of Q_B is thought to lie in this cleft when the plastoquinone molecule is bound at the Q_B site [12]. It was anticipated that a phenylurea type inhibitor with a hydrophobic trans stilbene moiety would not only show a higher binding affinity than the classic PS2 phenylurea inhibitor diuron (DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea) but would also have restricted movement in the binding site [13]. A novel stilbene urea, 3-(3-((E)2-(4-chlorophenyl)-ethenyl)phenyl-1,1-dimethylurea (chloro-PDU) did indeed have a high binding affinity [13]. In order to further characterize the binding of chloro-PDU to D1, photoaffinity labelling derivatives of this compound have been synthesised in which the 4-chloro group has been replaced by either an azido function (azido-PDU, 1) or a trifluoromethyl-diazirinyl group (diazirinyl-PDU, 2). The azido-derivative was synthesised for comparison with azidomonuron. In azidomonuron, the azido-function is relatively close to the urea moiety, whereas in the stilbenes made in this study, the photo-activatable

group is located on the distal phenyl ring somewhat remote from the urea group. It was thus hoped that by identifying amino acids labelled by these latter compounds, some idea of the orientation of these molecules in the binding cleft could be deduced by relating this information to the modelling studies.

A major deficiency of azido-compounds as photoaffinity reagents is the relatively poor reactivity of the nitrene photoproduct. Normally-substituted nitrenes will only react with N-H, S-H, S-CH_3, C=O and O-H of the groups usually present in protein binding sites [14,15]. This potential selectivity can lead to non-specific reaction of photoaffinity probes in which more reactive groups distant from the true binding site are selectively and artifactually labelled, particularly if the lifetime of the reactive photoproduct is long (see also [16]). An additional potential short-coming is the fact that the adducts which are formed between nitrenes and carboxyl groups (amides) are unstable to sequencing by the Edman degradation method. Diazirines have the advantage over azides in that they give rise to highly reactive carbenes of short half-life. Carbenes are also capable of reacting with C-H groups and are thus particularly suited for labelling hydrophobic regions of proteins [17]. Generally, the adducts are more stable than those formed by nitrenes and it is reported that the carbenes produced upon photoactivation of diazirines form stable covalent linkages to proteins with the exception of unstable bonds to Glu, Asp, Gln and Asn residues [17].

RESULTS AND DISCUSSION

Synthesis of azido-PDU (1) (Schemes 1(a) and 1(b))

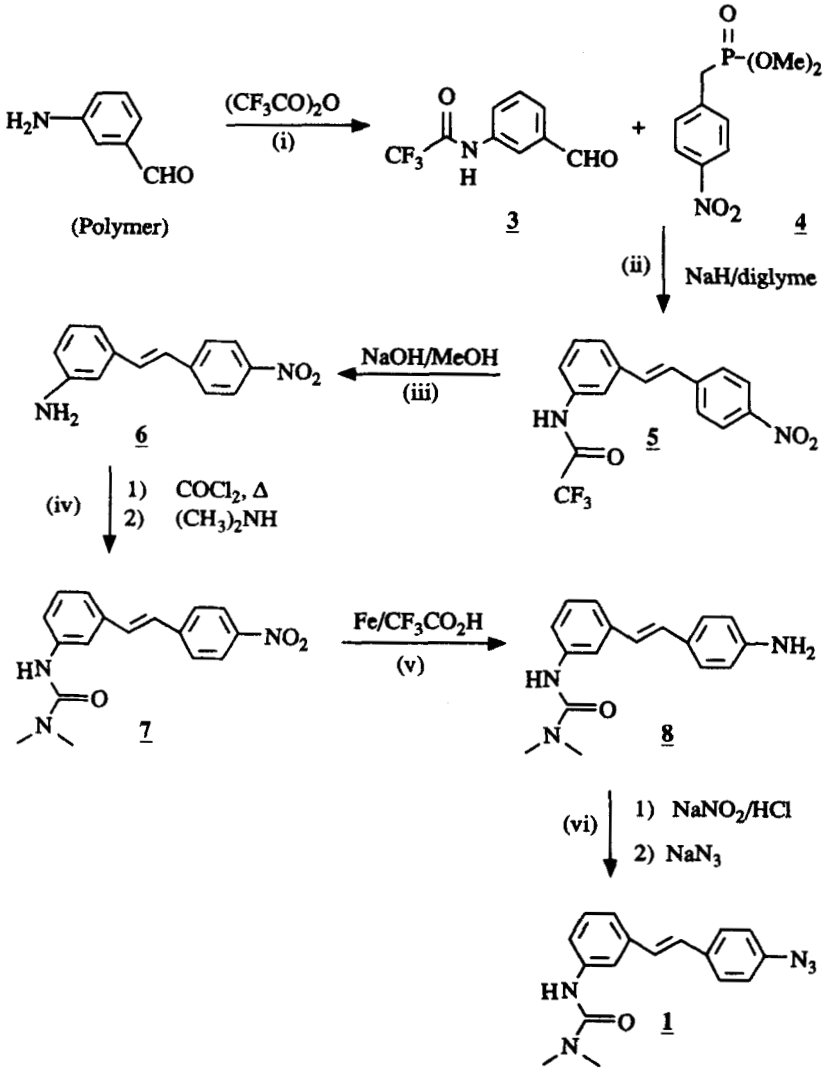
One of the problems concerned with the synthesis of 1 relates to the synthesis of the anilino precursor of the azido group, the latter being introduced via substitution of the aryl diazonium salt by azide. Synthesis of the urea moiety on the other phenyl ring could not have been effected via a nitro aniline, since the nitro group could not have been reduced once the azido function was introduced because all reagents which reduce nitro will also reduce azido. The problem was solved by introducing the urea via a trifluoroacetyl-protected anilino function by the trifluoroacetylation of 3-aminobenzaldehyde polymer. The stilbene entity was synthesised by a Wadsworth-Emmons reaction and mostly gave exclusively E isomers, as is evidenced by the ^{1}H NMR spectra of the ethene protons, in contrast to the more conventional triphenyl phosphonium ylid-mediated Wittig reactions which gave E/Z mixtures. An

exception was in the synthesis of 22, when the reaction led to a proportion of the Z isomer(see Scheme 2 below).

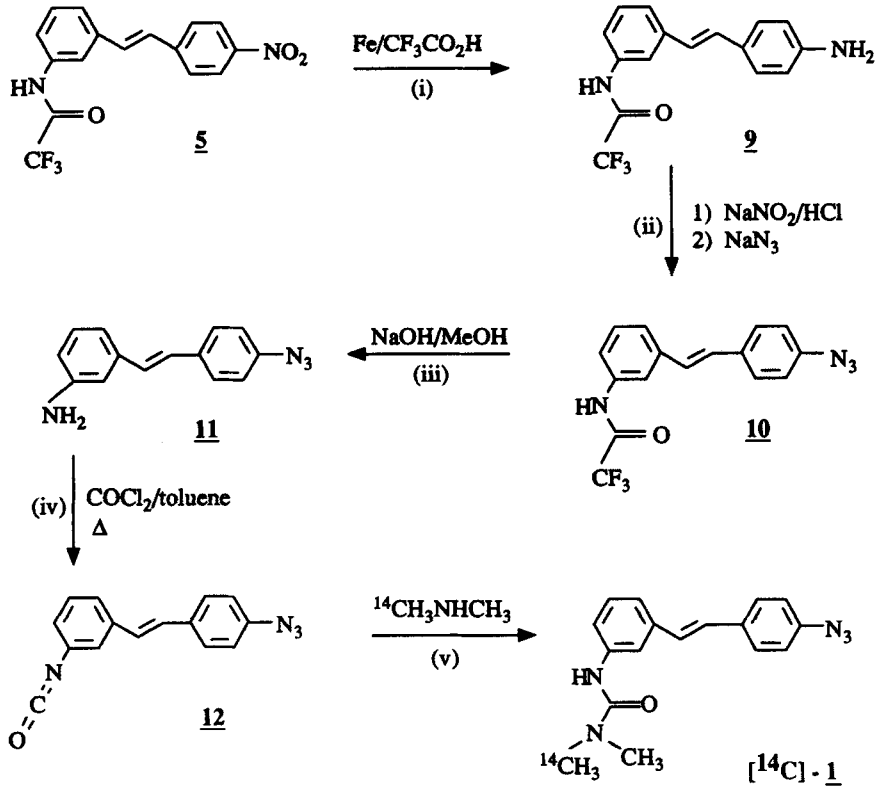
The synthesis of non-radiolabelled 1 is shown in Scheme 1(a). In this, the dimethyl urea function was synthesised prior to the reduction of the nitro group and the introduction of the azido group. The dimethyl urea group thus became a protecting group for the anilino function.

In synthesising a radiolabelled form of 1, it was clearly advantageous if the radiolabel could be introduced late in the synthesis scheme, in this

Scheme 1(a) - Synthesis of azido-PDU (1)



Scheme 1(b) - Synthesis of[^{14}C]-azido-PDU (1)



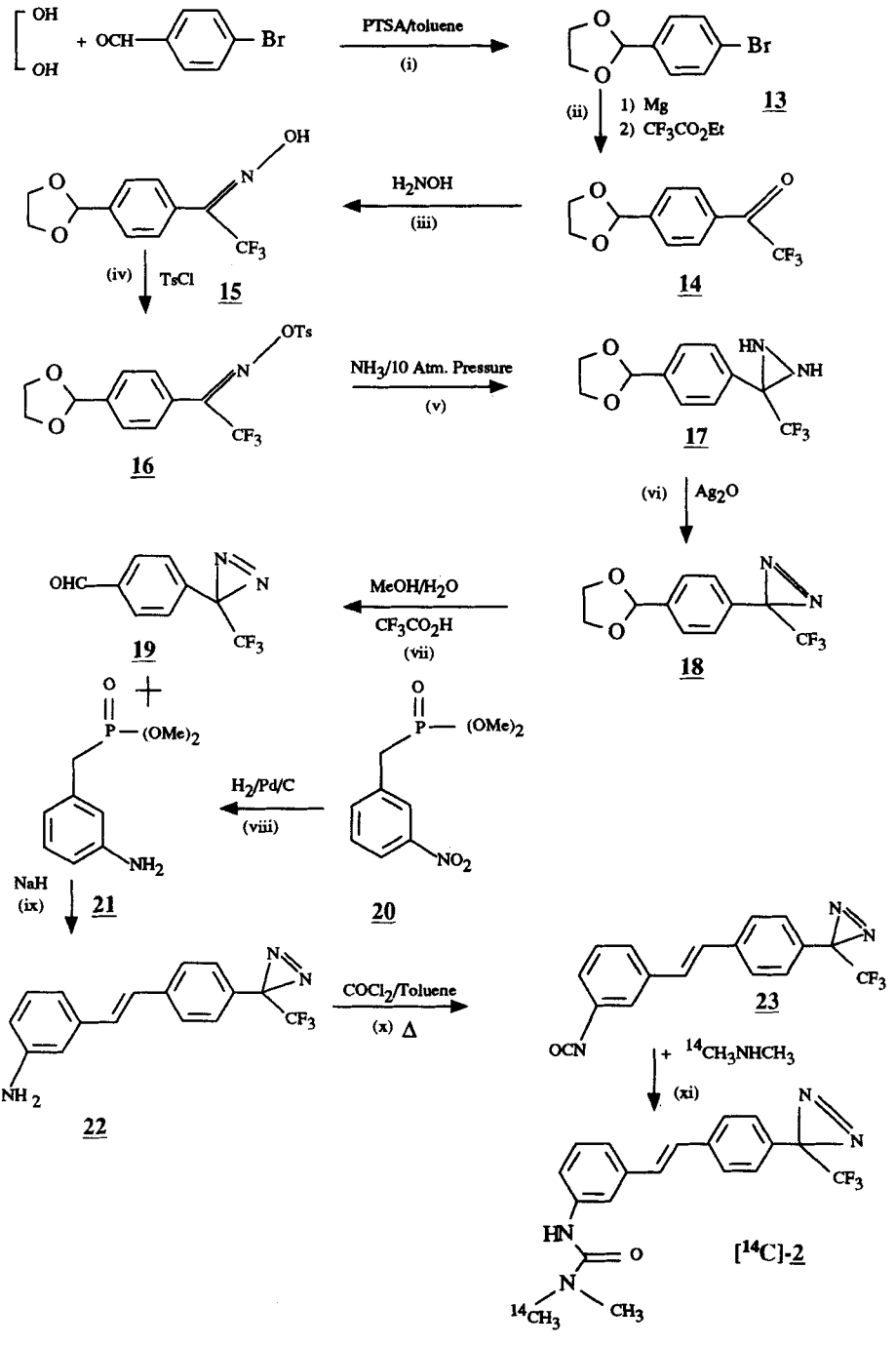
case by the reaction of [^{14}C]-dimethylamine with the isocyanate. Thus, the azido-substituted trifluoroacetylated amine was synthesised rather than the analogous urea as in Scheme 1 (a) and the dimethyl urea was synthesised at the last step. Iron in trifluoroacetic acid was found to be much superior to Pd/C with a hydrogen transfer reagent for the reduction of nitrostilbenes to the analogous anilines. The latter reduction systems always gave some reduction of the alkene function.

Synthesis of diazirinyl-PDU (2) (Scheme 2)

The initial synthesis scheme for the diazirine stilbene photoaffinity probe (2, diazirinyl-PDU) was based on the synthesis of 3-trifluoromethyl-3-(4-bromomethylphenyl)diazirine as an intermediate. In this synthesis route, 2,2,2-trifluoro-1-(4-methylphenyl)1-ethanone was synthesised from 4-bromotoluene and ethyl trifluoromethylacetate via a Grignard reaction [18]. This intermediate was converted to 3-trifluoromethyl-3-(4-methylphenyl)diazirine by the same route as

shown for steps (iii) - (vi) in Scheme 2. However, attempts to produce 3-trifluoromethyl-3-(4-bromomethylphenyl)diazirine by bromination of the 3-trifluoromethyl-3-(4-methylphenyl)diazirine with reagents such as N-bromosuccinimide/dibenzoyl peroxide met with no success.

Scheme 2 - Synthesis of [^{14}C]-diazirinyl-PDU (2)



This problem was circumvented by adopting the route shown in Scheme 2. In this route, the diazirinyl function was constructed on the aldehyde moiety, rather than the proto-phosphonium portion (cf Scheme 1(a)). The aldehyde function of 4-bromobenzaldehyde was protected as the 1,3-dioxolane and the 3-trifluoromethyl diazirinyl group synthesised via the oxime, 15, and tosyl oxime, 16, using the general method of Nassal [19]. Reaction of the latter with ammonia under pressure gave 17, presumably via a hydrazide intermediate. Oxidation of 17 with silver oxide then gave the substituted diazirine, 18.
One problem with the synthesis involved isolation of the trifluoromethyl ketone, 14 as a by-product in the silver oxide oxidation of the diazirine, 17. Deprotection to form the aldehyde, 19, followed by a Wadsworth-Emmons reaction with 21 gave the diazirinyl substituted stilbene aniline, 22 as an E/Z mixture. Synthesis of the urea, 2, was then effected via synthesis of the isocyanate and reaction with [^{14}C]-labelled dimethylamine. The E and Z isomers were separated by preparative TLC.

EXPERIMENTAL

Analytical methods

TLC was carried out using silica gel plates (Merck) containing fluorescent indicator. Chromatographic purification was by flash chromatography using 250-400 mesh silica gel. Melting points are uncorrected and were measured on an electrically-heated microscope stage. IR spectra were measured using either a Nujol mull or a liquid film with a Pye Unicam SP2000. ^{1}H NMR spectra were measured at either 60 MHz using a Jeol PMX60 or at 300 MHz using a Nicolet QE300, both using tetramethylsilane as reference. Mass spectra were measured using a Finnigan Mat model 4500 with methane as ionizing gas.

Synthesis of azido-PDU (1)

2,2,2-trifluoro-N-(3-formylphenyl)ethanamide (3) Scheme 1(a), step (i)

3-Aminobenzaldehyde polymer (10.3 g, 0.1 mol) was refluxed with trifluoroacetic anhydride (25 ml) under dry nitrogen for 18 hours. The resultant solution was filtered, diluted with ethanol and evaporated in vacuo to yield a brown gum. The gum was dissolved in ethyl acetate and the organic phase extracted with saturated sodium hydrogen carbonate, 6M HCl and saturated NaCl. The organic phase was dried over sodium sulphate and evaporated to give a brown crystalline solid (9.45 g). This was purified by vacuum sublimation at 110°C, 0.1 mm and finally

recrystallised from toluene to yield the product as white needles, Mpt 83-85°C, yield 6.07 g, (28%).
Analysis: Calculated for $C_9H_6F_3NO_2$: C, 49.8 : H, 2.8 : N, 6.5. Found: C, 49.9 : H, 2.6 : N, 6.3.

Dimethyl(4-nitrophenylmethyl)phosphonate (4)
4-Nitrobenzyl bromide (10.8 g, 0.05 mol) was dissolved in dry toluene (30 ml). Trimethyl phosphite (8.5 ml, 8.94 g, 0.072 mol) was added and the reaction refluxed for 24 hours under nitrogen. The solvent was removed in vacuo and the product purified by vacuum sublimation (150°C, 0.05 mm) and flash chromatography (ethyl acetate:ethanol, 2:1,v/v) gave the product as a pale yellow solid, Mpt. 72-77°C, yield 8.20 g (67%).
Analysis: Calculated for $C_9H_{10}NO_5P$: C, 44.5 : H, 4.2 : N, 5.8. Found: C, 44.4 : H, 4.7 : N, 5.8. M^+, 243.

2,2,2-Trifluoro-N-(3-((E)-2-(4-nitrophenyl)ethenyl)phenyl)-ethanamide (5), Scheme 1(a), step (ii)
Sodium hydride (0.50 g, 60%, 1.25×10^{-2} mol) was freed of oil by using dry n-pentane and stirred in dry 1,2-dimethoxyethane (30 ml) under dry nitrogen. 4 (2.43 g, 1.0×10^{-2} mol) was added and allowed to react for 1 hour at room temperature to produce the bright red carbanion. 3 (2.17 g, 1.0×10^{-2} mol) was dissolved in dry dimethoxyethane (10 ml) and added to the above solution over 5 min. After ten days the solvent was removed and the residue dissolved in ethyl acetate and filtered through Celite. The filtrate was washed with sodium bisulphite solution, sodium hydrogen carbonate and brine, dried and the solvent removed to yield 2.47 g bright yellow crystals. Final purification was by recrystallisation from toluene to give 1.52 g 5 (45%). Mpt 165-168°C.
Analysis: Calculated for $C_{16}H_{11}N_2O_3F_3$: C, 57.1 : H, 3.3 : N, 8.3. Found: C, 57.2 : H, 3.4 : N, 8.4. M^+, 336. ^1H NMR (300 MHz, $CDCl_3$) δ 7.20 (2H, dd J = 15 Hz).

3-(2-(4-nitrophenyl)ethenyl)aniline (6), Scheme 1(a), step (iii)
5 (1.0 g, 3.0×10^{-3} mol) was dissolved in ethanol (20 ml). Sodium hydroxide (0.5 g) in water (2 ml) was added and the solution refluxed for 3 hours. During refluxing a yellow solid (product) separated. The reaction was cooled, diluted with water (100 ml) and the product filtered. Yield, 0.698 g (98%) orange solid, Mpt 190-195°C.
Analysis: Calculated for $C_{14}H_{12}N_2O_3$: C, 70.0 : H, 5.0 : N, 11.7. Found: C, 67.8 : H, 4.9 : N, 11.7. M^+, 240. ^1H NMR (300 MHz, $CDCl_3$) δ 7.38 (2H, dd J = 16 Hz).

1,1-Dimethyl-3(3-((E)2-(4-nitrophenyl)ethenyl)phenyl)urea (7), Scheme 1(a), step (iv)

Dry toluene (20 ml) was cooled to 0°C and saturated with phosgene. 6 (0.06 g, 2.5 x 10^{-3} mol) was added and the mixture refluxed for 1 hour with phosgene still bubbling. The reaction was then bubbled with dry nitrogen, and allowed to cool. Dimethylamine (5.0 ml) was added and the reaction stirred for 18 hours. The solvent was removed and the remaining yellow solid washed with water and filtered. Yield, 0.728 g yellow solid (97%), Mpt 180-186°C.

Analysis: Calculated for $C_{17}H_{17}N_3O_2$: C, 65.6 : H, 5.5 : N, 13.6. Found: C, 65.6 : H, 5.6 : N, 13.3. M^+, 311. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (2H, dd J = 16 Hz).

3-(3-((E)2-(4-aminophenyl)ethenyl)phenyl)-1,1 dimethylurea (8), Scheme 1(a), step (v)

7 (0.06 g, 1.93 x 10^{-3} mol) was dissolved in glacial acetic acid (20 ml). Iron powder (1.08 g, 1.93 x 10^{-2} g atom) was added and the mixture stirred under a nitrogen atmosphere for 21 hours. The mixture was filtered and the filtrate evaporated. The residue was dissolved in ethyl acetate and extracted with 10% (w/v) potassium carbonate. The organic layer was dried and evaporated to give a pale yellow solid, Mpt 125-127°C. Yield, 0.466 g (86%).

Analysis: Calculated for $C_{17}H_{19}N_3O$: C, 72.6 : H, 6.8 : N, 14.6. Found: C, 75.8 : H, 7.1 : N, 15.0. M^+, 281.

3-(3-((E)2-(4-azidophenyl)ethenyl)phenyl)-1,1-dimethylurea (1), Scheme 1(a), step (vi)

8, (0.366 g, 1.3 x 10^{-3} mol) was slurried in 1 M HCl (5.0 ml) and cooled to 0°C. Sodium nitrite (0.11 g, 1.6 x 10^{-3} mol) was added and stirred for 1 hour. The reaction was filtered. Sodium azide (0.65 g, 0.01 mol) was added to the filtrate. The pink/cream solid which separated over 15 min was filtered, washed with water and dried. Purification was by flash chromatography (toluene:acetone, 3:1,v/v) and recrystallisation from toluene. Final yield, 0.125 g (32%) cream solid, Mpt 148-150°C.

Analysis: Calculated for $C_{17}H_{15}N_4O$: C, 66.9 : H, 5.0 : N, 22.9. Found: C, 66.1 : H, 5.6 : N, 22.6. M^+, 305.

IR (mull) 2140 cm^{-1} (N_3 stretch), 1645 cm^{-1} (CO stretch).

Synthesis of[^{14}C]azido-PDU ([^{14}C]-1)

N-(3-((E)2-(4-aminophenyl)-ethenyl)phenyl)2,2,2-trifluoromethyl- ethanamide (9), Scheme)1(b) step (i)

5 (0.25 g, 7.4 x 10^{-4} mol) was dissolved in trifluoroacetic acid (5 ml) under nitrogen. Reduced iron powder (0.48 g, 8.6 x 10^{-3} g atom) was added and the mixture stirred for 4 hours. The solvent was removed and the residue dissolved in ethyl acetate. The organic phase was extracted with saturated sodium hydrogen carbonate, dried over sodium sulphate and evaporated to yield a yellow solid. This was recrystallised from ethyl acetate to yield a pale cream solid. Yield, 0.192 g (85%), Mpt 208-212°C.
Analysis: Calculated for $C_{16}H_{11}F_3N_4O$: C, 62.7 : H, 4.2 : N, 9.1.
Found: C, 62.5 : H, 4.3 : N, 9.0. M^+, 306.
IR (Nujol mull) 3330 and 3400 cm^{-1} (NH stretch), 1717 cm^{-1} (CO stretch). ^1H NMR (300 MHz, $CDCl_3$). δ 11.40 (1H, s (NH)). δ 6.92 (2H, dd J = 17 Hz). δ 5.32 (2H, s (NH_2)).

N-(3-((E)2-(4-azidophenyl)ethenyl)phenyl)2,2,2-trifluoromethyl-ethanamide (10), Scheme 1(b), step(ii)
9 (102 mg, 3.3 x 10^{-4} mol) was stirred in 1 M HCl (1.0 ml) and cooled to 0°C. Sodium nitrite (28 mg, 4.0 x 10^{-4} mol) in water (1.0 ml) was added and the reaction stirred for 30 minutes. The yellow suspension was then added to a solution of sodium azide (0.1 g, 1.5 x 10^{-3} mol) in water. The pale yellow solid which separated was filtered and washed with water. It was essentially pure by TLC and used without further purification. Yield, 103 mg (94%), Mpt 134-138°C.
Analysis: Calculated for $C_{16}H_{11}F_3N_4O$: C, 57.8 : H, 3.3 : N, 16.9.
Found : C, 57.0 : H, 3.0 : N, 16.2. IR (Nujol mull) 3330 and 3400 cm^{-1} (NH stretch), 1710 cm^{-1} (CO stretch). ^1H NMR (300 MHz, $CDCl_3$). δ 11.40 (1H, s (NH)) . δ 6.92 (2H, dd J = 17 Hz). δ 5.32 (2H, s (NH_2)).

E-3-(2-(4-azidophenyl)ethenyl)aniline (11), Scheme 1(b), step_(iii)
10, (83 mg, 2.5 x 10^{-4} mol) was dissolved in ethanol (5 ml). Sodium hydroxide solution (1.0 M, 1.0 ml, 1.0 x 10^{-3} mol) was added and the mixture refluxed under nitrogen for 30 minutes. Water was added (ca. 3 ml) to the refluxing solution which was then allowed to cool. The solid which separated was filtered, washed with water and dried to give 50 mg (85% yield) of a pale yellow solid, Mpt 140-142°C.
Analysis: Calculated for $C_{14}H_{12}N_4$: C, 71.2 : H, 5.1 : N, 23.7. Found: C, 74.9 : H, 5.3 : N, 24.7. IR (Nujol mull) 3350 and 3440 cm^{-1} (NH stretch) , 2130 cm^{-1} (N_3 stretch).

[^{14}C]-labelled 3-(3-((E)2-(4-azidophenyl)ethenyl)phenyl)1,1-dimethylurea ([^{14}C]-1), Scheme 1(b), steps(iv) and (v).
Dry toluene (5 ml) was cooled to 0°C and saturated with phosgene. 11, (20 mg, 8.5 x 10^{-5} mol) was added. After 10 minutes the reaction was

refluxed for a further 30 minutes with phosgene still being passed into it. The reaction was allowed to cool and purged with nitrogen to remove excess phosgene. The solvent was removed to give the isocyanate derivative, 12 in 77% yield.
IR (Nujol mull) 2320 and 1597cm^{-1} (NCO stretch) , 2130 cm^{-1} (N_3 stretch).

Di[^{14}C]methylamine hydrochloride (Amersham, batch 46, 58 Ci mol^{-1}, 500 μCi, 8.5 x 10^{-5} mol) in ethanol (2.0 ml) was stripped of solvent. The residue was resuspended in dry toluene (2.0 ml) and triethylamine (100 μl) in a 5 ml Reactivial. The isocyanate (12) (2.5 mg, 9.5 x 10^{-6} mol) in dry toluene (1.0 ml) was added and the reaction stirred in the dark for 18 hours. TLC of the reaction (toluene:acetone, 2:1,v/v) indicated one major UV absorbing spot at Rf 0.35, coincident with the urea, 1. The solvent was removed and the radiolabelled urea purified by preparative TLC using toluene:acetone, 2:1,v/v. The final purified compound weighed 2.4 mg, total radioactivity, 454 +/- 9 μCi (91% radiochemical yield) and was stored as a 3.9 x 10^{-3} M solution in ethanol. Sp. activity 58 Ci mol^{-1}.

Synthesis of diazirinyl-PDU (2)

2-(4-Bromophenyl)-1,3-dioxolane (13), Scheme 2, step (i)
4-Bromobenzaldehyde (15.0 g, 0.081 mol), 4-toluene sulphonic acid (0.2 g) and ethylene glycol (8.0 g, 0.13 mol) were dissolved in toluene and the reaction refluxed through a soxhlet extractor containing 4A molecular sieve for 2 hours. The cooled reaction mixture was extracted with saturated sodium hydrogen carbonate, dried and evaporated to yield the product. Yield, 18.3 g (99%). This was used without further purification.
^{1}H NMR (300 MHz, $CDCl_3$) δ 5.78 (1H, s) δ 7.36, 7.52 (4H, dd).

2-(4-(2,2,2-Trifluoroethanoyl)phenyl)1,3-dioxolane (14),Scheme 2, step (ii)
Magnesium (2.0 g, 0.082 g atom) was stirred in dry tetrahydrofuran (50 ml) under nitrogen. 2-(4-Bromophenyl)-1,3-dioxolane (13) (18.3 g, 0.08 mol) in dry THF (50 ml), was added at such a rate as to maintain reflux. The resultant Grignard solution was filtered through glass wool and added dropwise to a solution of ethyl trifluoroacetate (12.0 g, 0.0845 mol) in THF (50 ml) maintained between -50°C and -65°C over 1 hour. The solution was allowed to warm to 0°C and quenched by the addition of saturated aqueous ammonium chloride (150 ml). Ether (400 ml) was added. The solution was filtered and the organic layer

separated. This was dried and evaporated to give a yellow oil which was purified by flash chromatography (toluene:ethyl acetate, 3:1,v/v) to yield the product as a yellow semi-solid which was used for the further stages of the synthesis without further purification.
^{1}H NMR (300 MHz, $CDCl_3$) δ 5.90 (1H, s) δ 7.68, δ 8.10 (4H, dd).

2-(4-(2,2,2-Trifluoroethanoximinoyl)phenyl)1,3-dioxolane (15), Scheme 2, step (iii)
This was prepared from the trifluoromethyl ketone (14) by the method of Nassal [19] on a 0.04 mol scale. The yield of unpurified product of the E/Z oxime mixture was 98%. This was used for the next stage without further purification.

2-(2-(1,1,1-Trifluoro)-(2-tosyloximino)phenyl)1,3-dioxolane (16), Scheme 2, step(iv)
This was prepared by the method of Nassal [19] on a 0.04 mol scale and purified by vacuum sublimation. The yield was 30% Mpt 45-100°C (E/Z mixture).
^{1}H NMR (60 MHz, $CDCl_3$) δ 2.50 (3H, s) δ 4.10 (4H, m) δ 5.85 (1H, s).

3-(4-(1,3-Dioxolan-2-yl)phenyl)3-(trifluoromethyl)diaziridine (17), Scheme 2, step (v)
Ammonia gas (50 ml) was condensed into a heavy-walled 250 ml flask cooled in dry ice. 16 (2.6 g, 6.25 x 10^{-3} mol) was dissolved in dry THF, cooled to -50°C and the solution added to the liquid ammonia. The flask was sealed and allowed to warm to room temperature. The reaction time was 70 hour. The flask was re-cooled to -78°C, opened and the ammonia allowed to evaporate upon warming. The solvent was removed, the residue dissolved in ether and the insoluble ammonium tosylate filtered. The filtrate was stripped and the residue purified by flash chromatography (toluene:ethyl acetate, 7:2,v/v) to yield the product as a white solid. Mpt 111-115°C. Yield, 1.148 g (71%).
Analysis: Calculated for $C_{11}H_{11}N_2O_2$ F : C, 50.8 : H, 4.3 : N, 10.8.
Found: C, 50.9 : H, 4.5 : N, 9.8. M^+, 260.
^{1}H NMR (60 MHz, $CDCl_3$) δ 4.06 (4H, s) 5.85 (1H, s) δ 7.60 (4H, dd).

3-(4-(1,3-Dioxolan-2-yl) phenyl)3-(trifluoromethyl)diazirine (18), Scheme 2, step (vi)
17 (0.764 g, 3.93 x 10^{-3} mol) was dissolved in dry ether. Freshly prepared silver oxide (1.2 g) and anhydrous magnesium sulphate (1.0 g) were added and the reaction stirred under nitrogen for 8 hours. The reaction solution was filtered through Celite and the solvent removed.

Purification was by flash chromatography (toluene:ethyl acetate, 9:1,v/v) to give the product as a yellow liquid (0.42 g, 55%). A major by-product was the trifluoromethyl ketone, 14.
Analysis: Calculated for $C_{11}H_9N_2O_2F_3$: C, 51.1 : H, 3.5 : N, 10.8.
Found : C, 51.5 : H, 3.2 : N, 10.0. M^+, 258.
1R (film) 2090 cm^{-1} (weak) (N=N stretch) No band at 1723 cm^{-1} (CO stretch).

3-(4-formyl)phenyl-3-(trifluoromethyl)diazirine (19), Scheme 2, step (vii)
The dioxolane, 18 (0.40 g, 1.55 x 10^{-3} mol) was dissolved in methanol (5.0 ml), water (2.0 ml) and trifluoroacetic acid (1.0 ml). The reaction was stirred for 18 hours. Ethyl acetate was added and the organic phase separated, washed with sodium hydrogen carbonate, dried and evaporated to yield the product as a volatile liquid. Yield, 0.175 g (53%).
^{1}H NMR (50 MHz, $CDCl_3$) δ 10.02 (1H, s) δ 7.90, 7.35 (4H, dd J = 16 Hz).
M^+, 214.

Dimethyl(3-nitrophenylmethyl)phosphonate (20)
3-Nitrobenzyl bromide (15.2 g, 0.070 mol) was dissolved in dry toluene (80 ml). Trimethylphosphite (9.2 ml, 9.68 g, 0.079 mol) was added and the reaction refluxed under nitrogen for 21 hours. The solvent was removed and the product purified by flash chromatography (ethyl acetate:ethanol, 2:1,v/v) and by re-crystallization to give the product as a very pale yellow solid, Mpt 54-56°C, yield, 11.31 g (63%).
Analysis: Calculated for $C_9H_{12}NO_5P$: C, 44.1 : H, 4.9 : N, 5.7. Found : C, 44.8 : H, 5.2 : N, 6.6. M^+, 245.

Dimethyl(3-aminophenylmethyl)phosphonate (21), Scheme 2, step (viii)
20 (5.0 g, 0.020 mol) was hydrogenated in methanol (50 ml) in the presence of palladium on charcoal (0.5 g, 10%) with hydrogen (44 psi) for 1 hour. The reaction was filtered through Celite and the solvent removed. The product was washed with ether, dissolved in ethyl acetate and filtered to remove a small amount of contaminant. Removal of the solvent gave the product as a pale yellow oil, yield 4.14 g (95%).
Analysis: Calculated for $C_9H_{12}NO_5P$: C, 50.2 : H, 6.6 : N, 6.5. Found : C, 50.2 : H, 6.7 : N, 6.3. M^+, 215.

3-(4-(2-(3-aminophenyl)ethenyl)phenyl)3-(trifluoromethyl)-diazirine (22) Scheme 2, step (ix)
Sodium hydride (0.12 g, 60%, 3.0 x 10^{-3} mol) was freed of oil and stirred in dry 1,2-dimethoxyethane (5.0 ml). 21 (0.50 g, 2.35 x 10^{-3}

mol) in 1,2-dimethoxyethane (5.0 ml) was added and allowed to react for 30 minutes. 3.2 ml of this solution (7.5×10^{-4} mol) was added to the aldehyde 19 and allowed to react for 18 hours. The reaction was diluted with ethyl acetate (10 ml) and the gelatinous solid filtered. The solvent was removed and the residue purified by flash chromatography (toluene:ethyl acetate, 19:1,v/v) to give the product as a solid, Mpt 84-89°C. Yield 51 mg (24%).
^{1}H NMR (60 MHz, $CDCl_3$) δ 3.72 (1H, broad s) δ 6.80, 6.95 (E, J = 15 Hz) δ 6.52, 6.68 (Z, J = 10 Hz).

3-(3-(2-(4-(3-Trifluoromethyldiazirin-3-yl)phenyl)ethenyl)-phenyl)1,1-dimethylurea (2), Scheme 2, steps (x) and (xi).
Dry toluene (5.0 ml) was saturated with phosgene at 0°C. 22 (20 mg, 6.6×10^{-5} mol) was added and the solution refluxed, still with phosgene being bubbled through for 1 hour. The reaction was cooled, purged with nitrogen and the solvent removed to give the isocyanate 23 in quantitative yield. Excess dimethylamine (1.0 ml) was added to 5.9×10^{-5} mol of the isocyanate in dry toluene (4.4 ml) and allowed to react at room temperature for 4 hours. The solvent was removed to give a cream solid. Purification was by flash chromatography (toluene:acetone, 3:1,v/v) to give the product as a solid, Mpt 126-134°C.
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.02 (2H, dd) δ 6.40 (1H, broad s) δ 3.0 (6H, s).

[^{14}C]-labelled 3-(3-((E)2-(4-(3-(trifluoromethyl)diazirin-3-yl)-phenyl)ethenyl)phenyl)1,1-dimethylurea ([^{14}C]-2)
Di[^{14}C]methylamine hydrochloride (Amersham, batch 46, 58 Ci mol^{-1}, 2.0 ml, 500 mCi, 8.62×10^{-6} mol) was freed of solvent under a stream of nitrogen. Finely ground anhydrous potassium carbonate (10 mg) was added, followed by the isocyanate (23) (8.0×10^{-6} mol) in toluene (600 ml). This was allowed to react for 18 hours. TLC (toluene:acetone, 3:1,v/v) followed by visualisation using the AMBIS beta scanner indicated a double product spot coincident with the pattern of the non-radiolabelled compound, Rf 0.30 (E) and Rf 0.28 (Z).

These products were purified by preparative TLC to give the E product, total radioactive yield 97.3 μCi, 58 Ci mol^{-1}, 1.68×10^{-6} mol and the Z product, radioactive yield, 43.4 μCi, 58 Ci mol^{-1}. The overall radiochemical yield was 140.7 μCi (28%).

REFERENCES

1. Velthuys, B.R.-FEBS Lett. 126: 277 (1981)
2. Vermaas, W.F.J., Arntzen, C.J., Yu, L.-Q and Yu, C.-A.- Biochim. Biophys. Acta 723: 266 (1983)
3. Kyle, D.J.- Photochem. Photobiol. 41: 107 (1985)
4. Mattoo, A.K., Hoffmann-Falk, H., Marder, J.B. and Edelman, M.- Proc. Natl. Acad. Sci. USA 81: 1380 (1984)
5. Pfister, K., Steinback, K.E., Gardner, G. and Arntzen, C.J.- Proc. Natl. Acad. Sci. USA 78: 981 (1981)
6. Michel, H., Epp, O. and Deisenhofer, J.- EMBO J. 5: 2445 (1986)
7. Michel, H and Deisenhofer, J. - Biochemistry 27: 1 (1988)
8. Trebst, A., Donner, W. and Draber, W.- Z. Naturforsch. 39c: 405 (1984)
9. Camilleri, P., Barker, M.D., Bowyer, J.R., Kerr, M.W. and Whitehouse, M.K. J. Agric. Food Chem.- 37: 1509 (1989)
10. Huppatz, J.L. and Phillips, J.N.- Z. Naturforsch. 42c: 679 (1987)
11. Yoneyama, K., Konnai, M., Honda, I., Yoshida, S., Takahashi, N., Koike, H. and Inoue, Y.- Z. Naturforsch. 45c: 317 (1990)
12. Robinson, H.H. and Crofts, A.R.- Biophys. J. 51: 125a (1987)
13. Bowyer, J.R., Hilton, M., Whitelegge, J., Jewess, P., Camilleri, P., Crofts, A. and Robinson, H.- Z. Naturforsch. 45c: 379 (1990)
14. Bayley, H. and Staros, J.V.- in "Azides and Nitrenes" (E.F.V. Scriven, ed.):433-490, Academic Press, New York,1984
15. Bayley, H. and Knowles, J.R.- Methods in Enzymology 46: 69 (1977)
16. Gressel, J.- Plant Sci. Lett. 25: 99 (1982)
17. Bayley in "The Chemistry of Diazirines", Vol. 2 (Liu, M.T.H. ed.): 75-99, CRC Press, Boca Raton, 1987
18. Creary, X.- J. Org. Chem. 52: 5026 (1987)
19. Nassal, M.- Liebigs Ann. Chem. 1510 (1983)