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# Triazene Drug Metabolites. Part 17: Synthesis and Plasma Hydrolysis of Acyloxymethyl Carbamate Derivatives of Antitumour Triazenes

Emília Carvalho,[a,*] Ana Paula Francisco,[a] Jim Iley[b] and Eduarda Rosa[a,*]

[a]*Centro de Estudos de Ciências Farmacêuticas (CECF), Faculdade de Farmácia, Universidade de Lisboa, Avenida das Forças Armadas, 1600 Lisbon, Portugal*

[b]*Chemistry Department, The Open University, Milton Keynes, MK7 6AA, UK*

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**Abstract**—A series of 3-acyloxymethyloxycarbonyl-1-aryl-3-methyltriazenes **5** was synthesised by the sequential reaction of 1-aryl-3-methyltriazenes with (i) chloromethyl chloroformate, (ii) NaI in dry acetone, and (iii) either the silver carboxylate or the carboxylic acids in the presence of silver carbonate. The hydrolysis of these compounds was studied in pH 7.7 isotonic phosphate buffer and in human plasma. Triazene acyloxycarbamates demonstrated their ability to act as substrates for plasma enzymes. For compound **5f**, a pH–rate profile was obtained which showed the hydrolysis to involve acid–base catalysis. The reaction is also buffer catalysed. Thus, at pH 7.7, pH-independent, base-catalysed and buffer-catalysed processes all contribute to the hydrolysis reaction. The sensitivity of the hydrolysis reaction to various structural parameters in the substrates indicates that hydrolysis occurs at the ester rather than the carbamate functionality. In plasma, the rates of hydrolysis correlate with partition coefficients, the most lipophilic compounds being the most stable. An aspirin derivative suffers two consecutive enzymatic reactions, the scission of the aspirin acetyl group being followed by the scission of the acyloxy ester group. These results indicate that triazene acyloxymethyl carbamates are prodrugs of the antitumour monomethyltriazenes. They combine chemical stability with a rapid enzymatic hydrolysis, and are consequently good candidates for further prodrug development. Moreover, this type of derivative allowed the synthesis of mutual prodrugs, associating the antitumour monomethyltriazenes with anti-inflammatory NSAIDs as well as with the anticancer agent butyric acid. 

## Introduction

For many years dacarbazine **1** (Fig. 1) has been one of the most widely used drugs to treat malignant melanoma. The incidence of this disease has increased recently and is expected to continue in the near future.[1]

The biological action of dacarbazine and, in general, the anticancer 1-aryl-3,3-dimethyltriazenes (**2**, in Fig. 2) is a consequence of their capacity to alkylate DNA. These compounds suffer metabolic oxidation by cytochrome P450 enzymes to give hydroxymethyltriazenes **3**, which, by loss of formaldehyde, generate the cytotoxic monomethyltriazenes **4**. These are known alkylating agents, capable of methylating DNA and RNA (Fig. 2).[2,3] With the objective of finding suitable prodrugs that by-pass the need for oxidative metabolism,[4] various derivatives of **3** and **4** have been synthesised, such as esters,[5] ethers,[6] thioethers,[7] and acyl derivatives.[8,9] None of these possess ideal prodrug characteristics. More recently, an imidazotetrazine — temozolomide — has been found to be active against several tumours in phase I clinical studies. Its activity is thought to be due to the generation of an alkylating monomethyltriazene by reaction with water.[10] Anticancer triazenes containing amino acid carriers have also been studied.[11,12] One such compound, an *N*-acetylalanyl derivative, displayed chemical stability in isotonic phosphate buffer yet rapid hydrolysis to the monomethyltriazene in plasma, indicating it to be a promising candidate for prodrug development.

As part of our ongoing work directed toward the development of triazene prodrugs, we have extended our investigations to encompass the acyloxymethyl carbamates **5** (Fig. 3). The acyloxymethyl carbamate functionality has been proposed as a promoiety for primary and secondary amines. Such compounds suffer esterase-catalysed hydrolysis of the ester function, producing an

*Corresponding author. Tel.: +351-217946477; fax: +351-21794670; e-mail: merosa@ff.ul.pt

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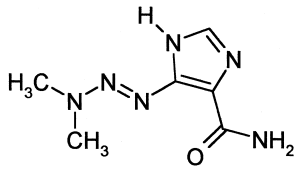

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**Figure 1.** Structure of dacarbazine.

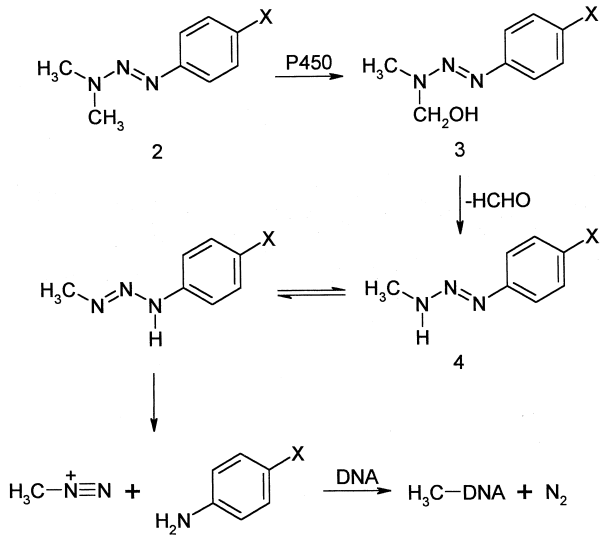


**Figure 2.** Oxidative demethylation pathway for 1-aryl-3,3-dimethyltriazenes.

unstable hydroxymethyl carbamate intermediate that liberates the amine drug and a carboxylic acid.[13,14] To assess the potential of compounds **5** as bioreversible prodrugs of the monomethyltriazenes **4**, we report the synthesis of some novel 3-(acyloxymethyloxycarbonyl)-1-aryl-3-methyltriazenes **5a**–**j** and an examination of their stability both in isotonic pH 7.7 phosphate buffer and plasma. The compounds synthesised use as carriers simple non-toxic carboxylic acids like acetic acid and *N*-acetylalanine, or other drugs containing a carboxylic acid group, forming mutual prodrugs.

## Results and Discussion

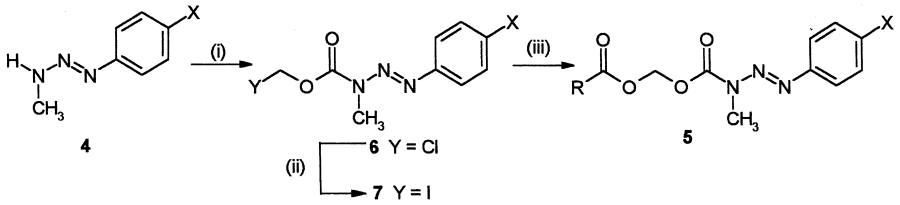
### Chemical synthesis

Synthesis of the desired compounds **5** proceeds via the 1-aryl-3-chloromethyloxycarbonyl-3-methyltriazenes **6** and the corresponding iodomethyl derivatives **7** (Fig. 3). The triazenes **6** themselves are efficiently synthesised by reaction of 1-aryl-3-methyltriazenes with chloromethyl chloroformate. Exchange of the Cl atom using NaI yields the corresponding 1-aryl-3-iodomethyloxycarbonyl-3-methyltriazenes **7**, which react in acetone in the presence of a tetrabutylammonium salt with either a silver carboxylate or its equivalent a carboxylic acid/silver carbonate. The nature of the solvent appears important for this latter reaction; in the synthesis of **5e**, when using tetrahydrofuran, we isolated the anhydride product of competitive attack of the nucleophile at the carbonyl group. Compounds **5** display the typical triazene *N*-methyl singlet in the $^1$H NMR spectra, ranging from δ 3.48 to 3.53 ppm. Compounds **5a**–**e** and **5g**–**i** exhibit an $OCH_2O$ methylene singlet ranging from 5.99 to 6.27 ppm. In compounds **5f** and **5j**, the $OCH_2O$ protons are diastereotopic and the signal for these appears as two doublets with $J = 5.6$ Hz.

### Kinetic studies

The hydrolysis of the triazene carbamate prodrugs **5** to the corresponding 1-aryl-3-methyltriazenes was studied both in isotonic phosphate buffer and in 80% human plasma containing 20% isotonic phosphate buffer. The hydrolysis of **5f** was also studied across the pH range 1–9 in aqueous buffers using several buffer concentrations at each pH value. Under these reaction conditions the monomethyltriazenes are also unstable and hydrolyse further to the corresponding anilines. These reactions were easily monitored by HPLC, following the loss of starting material.

Half-lives for the hydrolysis of triazenes **5** in isotonic phosphate buffer are given in Table 1. Inspection of Table 1 shows that they decompose at physiological pH with half-lives ranging from 80 min to 7 days. The most reactive of these, **5f**, was studied in more detail, and pseudo-first-order rate constants for its hydrolysis were determined in aqueous buffers (using several buffer concentrations at each pH) and in HCl solutions. The values of $k_{obs}$ obtained are dependent upon both the pH of the solution and the buffer concentration. Using the intercepts of plots of $k_{obs}$ versus buffer concentration, together with $k_{obs}$ values determined in HCl solutions, a pH–rate profile was constructed (Fig. 4). This shows that there are regions of acid- and base-catalysis as well as a pH-independent region between pH 2 and 5 across which the compound is most stable. The bimolecular rate constants for buffer catalysis, $k_B$, were determined using the slopes of plots of $k_{obs}$ versus buffer concentration (data not shown). Figure 5 shows the correlation between $k_B$ and $pK_a'$ (the p*K*a values corrected for ionic strength[15]) which is linear ($r^2 = 0.95$) and gives a Brønsted β value of 0.5. The pH–rate profile, together with the Brønsted plot for **5f**, reveals that at pH 7.7 the hydrolysis reaction comprises three components: pH-independent, base-catalysed and buffer-catalysed reactions. The major component appears to be the base-catalysed reaction. Thus, conclusions about the factors affecting the relative magnitudes of the $t_{1/2}$ values in phosphate buffer for the other compounds in Table 1 must be made with caution. Nevertheless, certain features are apparent. Comparison of **5a**–**d** reveals that the rate of reaction is only very slightly influenced by the nature of the substituent -X in the triazene aryl group, the more reactive compounds being those containing electron-attracting substituents. However, compared to the hydrolysis of acyltriazenes[8,11] this effect is very small. In contrast, comparison of compounds **5b,e,g** reveals the carboxylic acid moiety to have a much greater influence on reactivity,[16] the relative differences in rates paralleling the known differences in reactivity for the hydrolysis of ethyl esters.[17] These reflect the electronic and steric effects on the rate. Indeed, the



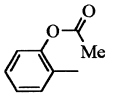
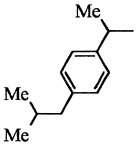

| | R | X |
|---|---|---|
| **5a** | $CH_3$– | CN |
| **5b** | $CH_3$– | COOEt |
| **5c** | $CH_3$– | Br |
| **5d** | $CH_3$– | $CH_3$ |
| **5e** | $CH_3CH_2CH_2$– | COOEt |
| **5f** | $CH_3(NHAc)CH$– | COOEt |
| **5g** | Ph– | COOEt |
| **5h** |  | COOEt |
| **5i** |  | COOEt |
| **5j** |  | COOEt |

**Figure 3.** Synthesis and structures of the triazenes **5**. Reagents: (i) $ClCH_2OCOCl$; (ii) NaI; (iii) $RCO_2Ag$ or $RCO_2H/Ag_2CO_3$.

**Table 1.** Half-lives, $t_{1/2}$, in both pH 7.7 isotonic phosphate buffer and 80% human plasma at 37 °C and log *P* values for the triazenes **5**[a]

| | | | $t_{1/2}$ (min) | |
|---|---|---|---|---|
| | Log $P_{exp}$ | Log $P_{calc}$ | pH 7.7 buffer | Plasma |
| **5a** | 2.08±0.02 | 2.29±0.61 | 358±7 | 5±0.2 |
| **5b** | 3.44±0.09 | 3.23±0.61 | 388±19 | 7±0.07 |
| **5c** | 3.64±0.05 | 3.37±0.63 | 505±35 | 12±1.1 |
| **5d** | 3.28±0.06 | 2.81±0.60 | 428±21 | 17±1.5 |
| **5e** | 4.51±0.25 | 4.40±0.61 | 664±79 | 15±0.3 |
| **5f** | 2.73±0.01 | 2.76±0.66 | 81±2 | 12±0.7 |
| **5g** | — | 5.25±0.61 | >7500[b] | 47±3.8 |
| **5h** | — | 4.37±0.61 | 5622±56 | 1±0.07[c] |
| **5i** | — | 5.29±0.62 | 6386±650 | 34±0.7 |
| **5j** | — | 7.34±0.6 | 9870±1300 | 125±6.25 |

[a]Values represent the means of at least 3 determinations ±S.D.
[b]Estimated value. Due to the insolubility of the compound, results are not precise.
[c]Half-life for the cleavage of the acetyl group in the aspirin moiety.

increased rate of **5f** over **5b** despite increased steric hindrance is almost certainly due to the known electron-withdrawing ability of an NHAc group to enhance the reactivity of an adjacent carbonyl group.[12] Moreover, the greater stability of **5j** results from the steric hindrance around the ester carbonyl.[16] The salicylate derivatives **5h,i** have similar reactivity to **5g**, reflecting their similar structures. These observations help to identify which of the two functional groups—the ester or the triazene carbamate—is hydrolysed first. In general, esters are more reactive toward hydrolysis than carbamates. However, we have observed previously that *N*-acyltriazenes have a reactivity similar to that of esters.[8,9] Therefore, triazene derived carbamates may be anticipated to display enhanced reactivity over simple carbamates. Even so, the sensitivity of the reaction to the acyl moiety and insensitivity to the structure of the triazene moiety implies that these compounds suffer ester hydrolysis. Moreover, the Brønsted β value is typical of ester hydrolysis.[16,17]

Blood serum and plasma contain a range of enzymes that catalyse the hydrolysis of esters and amides.[18] Consequently, we were interested in examining the efficiency of the hydrolysis of the acyloxymethyloxycarbonyltriazenes **5** to the cytotoxic monomethyltriazenes **4** in human plasma. The results in Table 1 clearly show that all compounds are substrates for the plasma enzymes. Indeed, most liberate the monomethyltriazenes in a few

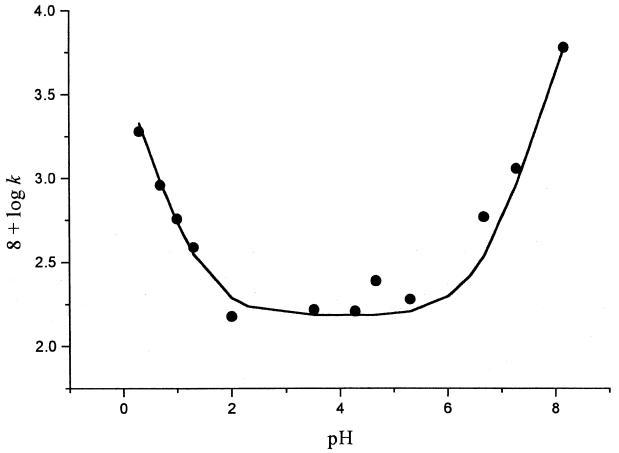



**Figure 4.** pH–Rate profile for the hydrolysis of **5f**; points are experimental, line is best fit using rate constants for the acid-, base- and uncatalysed hydrolyses of $4\times10^{-5}$ $M^{-1}$ $s^{-1}$, 42.3 $M^{-1}$ $s^{-1}$ and $1.55\times10^{-6}$ $s^{-1}$, respectively.

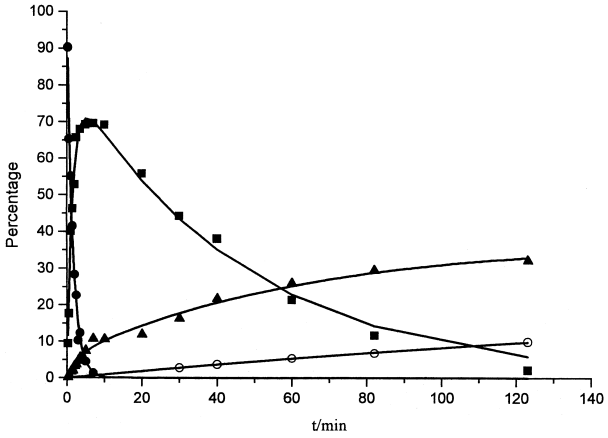



**Figure 6.** Time course for the formation and decay of intermediates in the plasma mediated cleavage of **5h**: ●, **5h**; ■, **5i**; ▲, **4**; ○, aniline (X = COOEt).

minutes. The rather short half-life observed for the aspirin derivative, **5h**, refers to the very fast enzymatic hydrolysis of the acetyl group which leads to liberation of the salicylic acid derivative **5i** (Fig. 6). As the reaction proceeds, the formation of both monomethyltriazene and aniline is also observed. However, due to the binding of **4** to plasma proteins[19,20] its quantitative measurement in serum proved difficult and complete mass balance was not possible.

There appears to be a superficial correlation of the plasma hydrolysis rates with those in pH 7.7 phosphate buffer despite the ca. 50-fold increase in the hydrolysis rates in plasma. For example, in the series **5a–d** electron-withdrawing groups in the aryl ring of the triazene moiety exert a slight rate enhancement; for **5b,e,j**, increasing steric bulk near to the ester carbonyl decreases the rate, the sterically hindered **5j** being the most stable compound studied; and, for **5b,g** the aroyl ester is less reactive than its alkanoyl counterpart. However, the enhanced chemical reactivity of **5f** is suppressed in plasma and, moreover, there is no apparent relationship between the reactivities of **5b, 5e** and **5f**. Indeed, a plot of $t_{1/2}$ (plasma) versus $t_{1/2}$ (buffer) (not shown) reveals no obvious correlation between the two.

The most striking correlation that encompasses all of the compounds studied (except **5i**, vide supra) is between prodrug stability (log $t_{1/2}$) and lipophilicity (log $P$) (eq 1). Elsewhere, it has been found that substrate lipophilicity is one of the main features that influences esterase activity, decreasing enzyme activity being associated with increasing lipophilicity.[21] However, bulky substituents in the substrate also influence enzyme activity. In the present case, the most lipophilic substrates are also the most sterically hindered and these effects cannot be separated.

$$\log t_{1/2} = 0.25\log P + 0.21 \quad (n = 9, r^2 = 0.89) \qquad (1)$$

## Conclusions

Dimethyltriazenes such as dacarbazine require metabolic demethylation to the corresponding cytotoxic monomethyltriazene by cytochrome P450 enzymes for them to display anticancer activity.[4] Indeed, there appears to be a direct correlation between the species-dependent extent of metabolism and anticancer activity, with humans exhibiting only a low level of metabolism.[4] Moreover, the peak plasma concentration of the active monomethyl metabolite is only ca. 0.6% of the overall drug dose.[4] Consequently, the current study demonstrates that the acyloxymethyl carbamate derivatives provide an alternative prodrug system for the

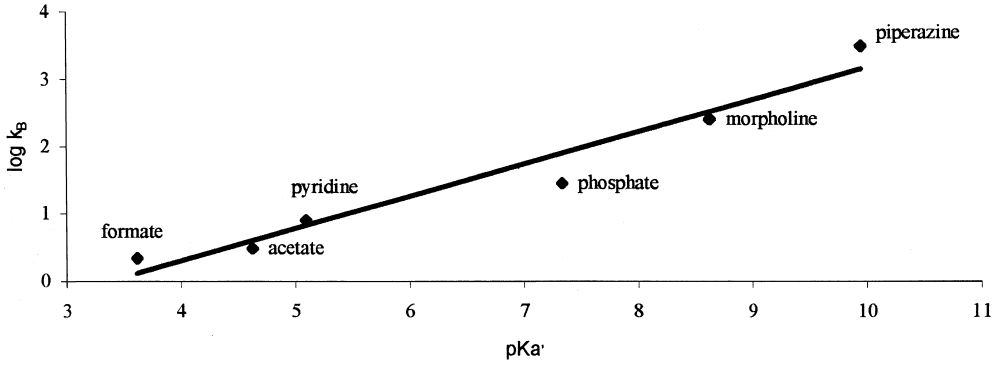



**Figure 5.** Brønsted plot for the buffer-catalysed hydrolysis of **5f**.

anticancer triazenes, one that by-passes the need for oxidative demethylation and allows for extensive and rapid transformation to the active metabolite. In addition, and in contrast to the dimethyltriazenes, a suitable choice of the acyloxy moiety in the carbamate prodrugs enables the half-life to be modified by between one and two orders of magnitude. The acyloxymethyl carbamate derivatives of the present study exhibit much better prodrug characteristics—chemical stability combined with enzymatic activation—than many of the systems studied previously. Thus, hydroxymethyltriazenes are too unstable in both aqueous media and plasma,[24] the derived ethers are stable chemically but themselves require oxidative metabolism to exert biological activity,[25] the corresponding thioethers appear not to be chemically or enzymically activated[7] and simple acyltriazenes are stable chemically and enzymically.[9] Only the aminoacyltriazenes have similar properties though they tend to have 4-fold larger plasma half-lives.[11] However, one of the benefits of the current strategy over the corresponding aminoacyl prodrug approach is that it enables the development of mutual prodrugs. For example, compounds **5h–j** are mutual prodrugs of anti-inflammatory agents which are often administered in conjuction with antitumour alkylating agents. Of more interest is compound **5e**, which is a mutual prodrug of butyric acid, a reported non-toxic natural product with antitumour activity.[22] Such prodrugs allow the possibility of simultaneously delivering two anticancer agents that function by different antitumour mechanisms. In this work the problem of specificity was not addressed. Nonetheless, it is possible to conceive that this strategy could include a promoiety that would confer site-selective cleavage. We are currently pursuing this strategy and also trying to extend the range of mutual prodrugs.

## Experimental

Melting points were determined in a Kofler camera Bock-Monoscop "M" and are uncorrected. IR spectra were recorded using a Perkin Elmer 1310 spectrophotometer. $^1$H NMR spectra were recorded using either a Jeol PMX-60 or a Jeol JNM-EX 400 spectrometer. Mass spectra were recorded using a VG Mass Lab 20-250 spectrometer. High-performance liquid chromatography (HPLC) was performed using a system consisting of a Merck-Hitachi lachrom L-7110 pump, a Spectra-Physics UV spectrachrom 100 detector, a Spectra-Physics SP4270 integrator, a Merck-Hitachi AS-2000 autosampler and a Lichrospher® 100 RP-8 (5 μm) column. Elemental analyses were obtained from Medac Ltd., Brunel Science Park, Englefield Green, Egham, Surrey, UK.

Plasma was derived from different healthy individuals, pooled, and kept at −18 °C until required. Ibuprofen, aspirin and salicylic acid were purchased commercially. All other chemicals were reagent grade except those used in kinetic and HPLC studies, which were analytical or LiChrosolv (Merck) grade, respectively. 1-Aryl-3-methyltriazenes **4** were synthesised by previously described methods.[23]

**General procedure for the synthesis of 1-aryl-3-chloromethyloxycarbonyl-3-methyltriazenes (6).** Chloromethyl chloroformate (5.8 mmol) was added slowly to a cold solution of the 1-aryl-3-methyltriazene (4.83 mmol), either in pyridine (10 mL) (X = CN, COOEt) or in dichloromethane (5 mL) containing triethylamine (5.8 mmol) (X = Br, Me). For the reactions in pyridine, the solution was stirred at room temperature, then poured onto ice and the resultant precipitate was dried. For the reactions in dichloromethane, the solution was evaporated under reduced pressure. The resultant solid from either method was recrystallised from diethyl ether/petroleum ether.

**3-Chloromethyloxycarbonyl-1-(4-cyanophenyl)-3-methyltriazene (6a).** Yield 84%; mp 117–8 °C; IR (KBr) 2224, 1761, 1136 cm$^{-1}$; $^1$H NMR δ 3.49 (3H, s, $NCH_3$); 5.98 (2H, s, $OCH_2Cl$); 7.76 (4H, s, Ar) ppm.

**3-Chloromethyloxycarbonyl-1-(4-ethoxycarbonylphenyl)-3-methyltriazene (6b).** Yield 86%; mp 86–7 °C; IR (KBr) 1719, 1741, 1278 cm$^{-1}$; $^1$H NMR δ 1.4 (3H, t, $CH_3$); 3.5 (3H, s, $NCH_3$); 4.35 (2H, q, $CH_2$); 5.95 (2H, s, $OCH_2Cl$); 7.65–7.68 and 8.05–8.08 (4H, AA′BB′, $J$ = 10 Ar) ppm.

**1-(4-Bromophenyl)-3-chloromethyloxycarbonyl-3-methyltriazene (6c).** Yield 82%; mp 101–3 °C; IR (KBr) 1735, 1479, 1124 cm$^{-1}$; $^1$H NMR δ 3.5 (3H, s, $NCH_3$); 6.0 (2H, s, $OCH_2Cl$); 7.6 (4H, s, Ar) ppm.

**3-Chloromethyloxycarbonyl-3-methyl-1-(4-tolyl)triazene (6d).** Yield 86%; mp 78–81 °C; IR (KBr) 1736, 1474, 1123 cm$^{-1}$; $^1$H NMR δ 2.45 (3H, s, $CH_3$); 3.57 (3H, s, $NCH_3$); 6.0 (2H, s, $OCH_2Cl$); 7.27–7.73 (4H, AA′BB′, $J$ = 8, Ar) ppm.

**General procedure for the synthesis of 1-aryl-3-iodomethyloxycarbonyl-3-methyltriazenes (7).** NaI (1.67 mmol) was added to a solution of the 1-aryl-3-chloromethyloxycarbonyl-3-methyltriazenes in dry acetone. The mixture was stirred at room temperature until completion of the reaction (ca. 18 to 24 h) as monitored by the disappearance of the $CH_2Cl$, and appearance of the $CH_2I$, peak in the $^1$H NMR. The solvent was removed under reduced pressure, the residue redissolved in dichloromethane, washed with saturated sodium bisulfite solution, dried and evaporated under reduced pressure. The resultant solid was used immediately without further purification.

**General procedure for the synthesis of 3-acyloxymethyloxycarbonyl-1-aryl-3-methyltriazenes (5).** The appropriate carboxylic acid silver salt (8.42 mmol), prepared by reaction of the sodium salt with silver nitrate, and tetrabutylammonium bromide (8.42 mmol) were added to a solution of **7** (8.42 mmol) in dry acetone (5 mL). The mixture was refluxed for ca. 10 h, filtered and evaporated under reduced pressure. The residue was subjected to chromatography (silica gel 60 H 77331, Merck) using petroleum ether/diethyl ether. The desired triazenes **5** were recrystallised from petroleum ether/diethyl ether. For compounds **5f,i,j**, the carboxylic acid and one equivalent of silver carbonate were used in place of the

silver carboxylate. In one case **5e** we attempted the above reaction in dry THF but isolated the anhydride ($\nu_{max}$ 1736, 1709, 1697, 1272, 1051 $cm^{-1}$; $\delta^H$ 1.03 (3H, t, $J=5$ Hz, Me), 1.27 (3H, t, $J=6$ Hz, Me), 1.7 (2H, m, $CH_2$), 2.93 (2H, t, $J=6$ Hz, $CH_2$), 3.5 (3H, s, N-Me), 4.40 (2H, q, $J=6$ Hz, $OCH_2$), 7.6–8.23 (4H, AA′BB′, $J=10$ Hz, Ar)). We did not investigate further the effect of solvent, but used dry acetone throughout.

**3-(Acetoxymethyloxycarbonyl)-1-(4-cyanophenyl)-3-methyltriazene (5a).** Yield 38%; mp 107 °C; IR (KBr) 2229, 1755, 1739, 1225 $cm^{-1}$; $^1H$ NMR δ 2.12 (3H, s, Ac), 3.51 (3H, s, NMe), 5.99 (2H, s, $OCH_2O$), 7.74 (4H, s, Ar) ppm; EIMS 276 ($M^+$), 160 ($ArN_3HCH_3^+$), 130 ($ArN_2^+$), 102 ($Ar^+$), 73, 43. Anal. calcd for CHNO: C, 52.17; H, 4.35; N, 20.29. Found: C, 52.4; H, 4.2; N, 20.4.

**3-(Acetoxymethyloxycarbonyl)-1-(4-ethoxycarbonylphenyl)-3-methyltriazene (5b).** Yield 65%; mp 84–6 °C; IR (KBr) 1740 br, 1717, 1275 $cm^{-1}$; $^1H$ NMR δ 1.4 (3H, t, $J=5$ Hz, Me), 2.1 (3H, s, Ac), 3.5 (3H, s, NMe), 4.35 (2H, q, $J=5$ Hz, $OCH_2$), 6.0 (2H, s, $OCH_2O$), 7.65–7.7 and 8.05–8.1 (4H, AA′BB′, $J=10$ Hz, Ar) ppm; EIMS 323 ($M^+$), 277 (M−EtOH), 177 ($ArN_2^+$), 149 ($Ar^+$), 43. Anal. calcd for CHNO: C, 52.01; H, 5.30; N, 12.99. Found: C, 51.9; H, 5.1; N, 12.7.

**3-(Acetoxymethyloxycarbonyl)-1-(4-bromophenyl)-3-methyltriazene (5c).** Yield 43%; mp 88–9 °C; IR (KBr) 1764, 1737 (br), 1138 $cm^{-1}$; $^1H$ NMR δ 2.16 (3H, s, Ac), 3.48 (3H, s, NMe), 5.99 (2H, s, $OCH_2O$), 7.52–7.57 (AA′BB′, $J=8.8$ Hz, Ar) ppm; EIMS 329/331 ($M^+$), 185/183 ($ArN_2^+$), 157/155 ($Ar^+$), 43. Anal. calcd for CHNO: C, 40.02; H, 3.66; N, 12.73. Found: C, 39.4; H, 3.7; N, 12.85.

**3-(Acetoxymethyloxycarbonyl)-3-methyl-1-(4-tolyl)triazene (5d).** Yield 44%; mp 52 °C; IR (KBr) 1769, 1739, 1370, 1133 $cm^{-1}$; $^1H$ NMR δ 2.16 (3H, s, Ac), 2.39 (3H, s, ArMe), 3.48 (3H, s, NMe), 5.99 (2H, s, $OCH_2O$), 7.25–7.56 (AA′BB′, $J=8.3$ Hz, Ar) ppm; EIMS 265 ($M^+$), 119 ($ArN_2^+$), 91 ($Ar^+$), 43. Anal. calcd for CHNO: C, 54.33; H, 5.70; N, 15.84. Found: C, 54.6; H, 5.7; N, 15.8.

**3-(Butanoyloxymethyloxycarbonyl)-1-(4-ethoxycarbonylphenyl)-3-methyltriazene (5e).** Yield 48%; mp 62 °C; IR (KBr) 1741 br, 1721, 1275 $cm^{-1}$; $^1H$ NMR δ 0.95 (3H, t, $J=5$ Hz, Me), 1.4 (3H, t, $J=5$ Hz, Me), 1.6–1.7 (2H, m, $CH_2^R$), 2.35 (2H, t, $J=8$ Hz, $COCH_2$), 3.45 (3H, s, NMe), 4.35 (2H, q, $J=5$ Hz, $OCH_2$), 6.0 (2H, s, $OCH_2O$), 7.6–7.65 and 8.05–8.1 (4H, AA′BB′, $J=10$ Hz, Ar) ppm; EIMS 351 ($M^+$), 306 (M−EtO), 177 ($ArN_2^+$), 149 ($Ar^+$), 71, 43. Anal. calcd for CHNO: C, 54.70; H, 6.02; N, 11.95. Found: C, 54.8; H, 6.0; N, 11.8.

**3-(2-(*N*-acetylamino)propanoyloxymethyloxycarbonyl)-1-(4-ethoxycarbonylphenyl)-3-methyltriazene (5f).** Yield 24%; mp 127–9 ° C; IR (KBr) 3295, 1743, 1718, 1656, 1276 $cm^{-1}$; $^1H$ NMR δ 1.42 (3H, t, $J=7$ Hz, Me), 1.45 (3H, d, $J=7.3$ Hz, α-Me), 2.04 (3H, s, Ac), 3.54 (3H, s, NMe), 4.41 (2H, q, $J=7.1$ Hz, $OCH_2$), 4.66–4.72 (1H, m, α-CH), 6.02–6.09 (2H, 2×d, $J=5.62$ Hz, $OCH_2O$), 5.97 (1H, d, $J=6.8$ Hz, NH), 7.67–7.69 and 8.11–8.13 (4H, AA′BB′, $J=8.55$ Hz, Ar) ppm; EIMS 394 ($M^+$), 349 (M−EtO), 177 ($ArN_2^+$), 149 ($Ar^+$), 86. Anal. calcd for CHNO: C, 51.78; H, 5.62; N, 14.20. Found: C, 51.8; H, 5.7; N, 14.2.

**3-(Benzoyloxymethyloxycarbonyl)-1-(4-ethoxycarbonylphenyl)-3-methyltriazene (5g).** Yield 49%; mp 108–10 °C; IR (KBr) 1742, 1740, 1718, 1272 $cm^{-1}$; $^1H$ NMR δ 1.42 (3H, t, $J=7.1$ Hz, Me), 3.53 (3H, s, NMe), 4.40 (2H, q, $J=6.8$ Hz, $OCH_2$), 6.27 (2H, s, $OCH_2O$), 7.48 (2H, t, $J=6.1$ Hz, $Ar^R$), 7.60 (1H, t, $J=7.3$ Hz, $Ar^R$), 7.68–8.11 (4H, AA′BB′, $J=8.5$ Hz, $Ar^X$), 8.14 (2H, d, $J=5.9$ Hz, $Ar^R$) ppm; EIMS 385 ($M^+$), 340 (M−EtO), 177 ($ArN_2^+$), 149 ($Ar^+$), 105 ($PhCO^+$). Anal. calcd for CHNO: C, 59.22; H, 4.97; N, 10.90. Found: C, 59.1; H, 4.9; N, 11.0.

**3-(2-Acetoxybenzoyloxymethyloxycarbonyl)-1-(4-ethoxycarbonylphenyl)-3-methyltriazene (5h).** Yield 46%; mp 124–5 °C; IR (KBr) 1745 (br), 1717 (br), 1606, 1276, 1206 $cm^{-1}$; $^1H$ NMR δ 1.42 (3H, t, $J=7.1$ Hz, Me), 2.39 (3H, s, Ac), 3.52 (3H, s, NMe), 4.41 (2H, q, $J=7.3$ Hz, $OCH_2$), 6.21 (2H, s, $OCH_2O$), 7.12 (1H, d, $J=8.3$ Hz, $Ar^R$), 7.35 (1H, t, $J=7.1$ Hz, $Ar^R$), 7.62 (1H, t, $J=5.9$ Hz, $Ar^R$), 7.68–7.70 and 8.10–8.13 (4H, AA′BB′, $J=8.3$ Hz, $Ar^X$), 8.12 (1H, d, $J=7.8$ Hz, $Ar^R$) ppm; EIMS 443 ($M^+$), 398 ($M^+$−EtO), 234 ($Ar^XN_3CO^+$), 177 ($Ar^XN_2^+$), 149 ($Ar^X$). Anal. calcd for CHNO: C, 56.88; H, 4.77; N, 9.48. Found: C, 56.8; H, 4.8; N, 9.4.

**1-(4-Ethoxycarbonylphenyl)-3-(2-hydroxybenzoyloxymethyloxycarbonyl)-3-methyltriazene (5i).** Yield 29%; mp 106–7 °C; IR (KBr) 3284, 1740, 1719, 1688, 1278 $cm^{-1}$; $^1H$ NMR δ 1.41 (3H, t, $J=7.1$ Hz, Me), 3.53 (3H, s, NMe), 4.39 (2H, q, $J=7.1$ Hz, $OCH_2$), 6.27 (2H, s, $OCH_2O$), 6.91 (1H, t, $J=7.6$ Hz, 5-H $Ar^R$), 7.0 (1H, d, $J=8.4$ Hz, 3-H $Ar^R$), 7.5 (1H, m, 4-H $Ar^R$), 7.66–7.69 and 8.09–8.12 (4H, AA′BB′, $J=8.5$, $Ar^X$), 7.92 (1H, d, $J=8.1$ Hz, 6-H $Ar^R$), 10.39 (1H, s, OH) ppm; EIMS 401 ($M^+$), 177 ($Ar^XN_2^+$), 149 ($Ar^X$), 121 ($Ar^RCO^+$). Anal. calcd for CHNO: C, 56.86; H, 4.77; N, 10.47. Found: C, 56.5; H, 5.0; N, 10.3.

**1-(4-Ethoxycarbonylphenyl)-3-(2-(4-isobutylphenyl)-propanoyloxymethyloxycarbonyl)-3-methyltriazene (5j).** Yield 60%; mp 59–61 °C; IR (KBr) 1746 (br), 1718, 1604, 1274 $cm^{-1}$; $^1H$ NMR δ 0.88 (6H, d, $J=6.8$, $^i$Pr), 1.43 (3H, t, $J=7.1$ Hz, Me), 1.53 (3H, d, $J=7.3$ Hz, Me), 1.78 (1H, m, $CH^iPr$), 2.39 (2H, d, $J=7.3$ Hz, $CH_2Ar^R$), 3.45 (3H, s, NMe), 3.78 (1H, q, $J=6.83$ Hz, CHCO), 4.41 (2H, q, $J=6.8$ Hz, $OCH_2$), 5.97–6.01 (2H, 2d, $J=5.62$ Hz, $OCH_2O$), 7.03–7.05 and 7.18–7.20 (4H, AA′BB′, $J=8.3$ Hz, $Ar^R$), 7.65–7.67 and 8.11–8.13 (4H, AA′BB′, $J=8.55$ Hz, $Ar^X$) ppm; EIMS 469 ($M^+$), 177 ($Ar^XN_2^+$), 161 ($Me_2CHArCHCH_3^+$), 149 ($Ar^{X+}$), 43. Anal. calcd for CHNO: C, 63.95; H, 6.65; N, 8.95. Found: C, 63.7; H, 6.7; N, 8.9.

## Hydrolysis studies

Compounds **5a**–**j** were incubated at 37 °C in 0.066 M pH 7.7 isotonic phosphate buffer. Compound **5f** was also studied at 37 °C in buffer solutions of different concentration

and pH. The ionic strength of these solutions was maintained at 0.2 M using $NaClO_4$. Samples of these reaction mixtures were analysed directly by HPLC. The 3-acyloxymethyloxycarbonyl-1-aryl-3-methyltriazenes were also incubated at 37 °C in 80% human plasma diluted with 0.066 M pH 7.4 isotonic phosphate buffer (the final pH of this mixture was 7.7). At appropriate intervals, samples of the plasma reactions were withdrawn, diluted with acetonitrile (0.4 mL) and centrifuged at 13,000 rpm for 5 min. The clear supernatant was analysed for substrate by HPLC. Quantitative analysis of the prodrugs and of their hydrolysis products was achieved by using a calibration curve constructed using standards chromatographed under the same conditions. For very fast reactions, e.g., those for **5h**, samples were withdrawn every 10 s and frozen at −18 °C prior to analysis. In all cases the HPLC conditions were: detector wavelength, 290 nm; mobile phase, acetonitrile:$H_2O$ (50:50 to 80:20). All reactions, both in buffers and in plasma, followed pseudo-first-order kinetics up to at least 4 half-lives.

## Partition coefficients

For compounds **5a**–**f**, partition coefficients were determined in octanol–pH 7.4 phosphate buffer at room temperature. Each phase was mutually saturated before the experiment. The compounds were dissolved in octanol and the octanol–pH 7.4 mixtures were shaken for 30 min to reach an equilibrium distribution; each phase was analysed separately by the HPLC method described above. The partition coefficients, $P_{exp}$, were obtained from the ratio of the peak area in octanol to the peak area in buffer. For compounds **5g**,**h**, the solubility in the aqueous medium was insufficient for an accurate determination of $P_{exp}$. Calculated values of $P$, expressed as log $P_{calc}$, were obtained using the software Chemsketch version 3.60 from Advanced Chemistry Development Inc.[26] For compounds **5a**–**f**, there is sufficiently good agreement between log $P_{calc}$ and log $P_{exp}$ for us to have confidence that the log $P_{calc}$ values for **5g**,**h** appropriately reflect the lipophilicity of these compounds.

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