between the charged head groups as well as chiral centers in the counterions are critical factors for determining the morphologies of the aggregates and their ability to gel various solvents.

Experimental Section

Compound **5** was synthesized as described before.[6] Compounds **1**–**4** and the CTA tartrates were prepared from **5** (dissolved in a water/methanol mixture) or cetyltrimethylammonium bromide by elution with water on a strongly basic anion exchange resin (Dowex 1 × 8, 50–100 mesh, 10 equivalents). The ammonium hydroxides were mixed with an equivalent amount of a tartaric acid, and the solution was lyophilized. The products were recrystallized from $CHCl_3/Et_2O$ (1/9, v/v) or $EtOH/Et_2O$ (1/9, v/v).

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2-Nitrobenzylarsonium Compounds That Photorelease Heavy-Atom Cholinergic Ligands for Time-Resolved Crystallographic Studies on Cholinesterases**

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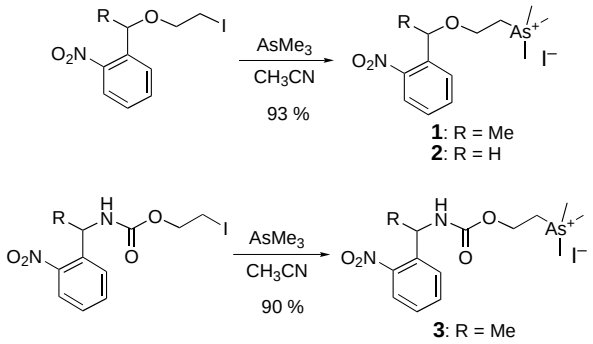
Heavy-atom derivatives have found widespread use in macromolecular crystallography, because they are essential in obtaining correct diffraction phases which are required for solving the three-dimensional structure of proteins.[1] Photolabile precursors of biologically interesting molecules, which can be converted rapidly from an inactive form to an active form by light (caged compounds), can provide temporally and spatially controlled release of enzyme substrates or receptor ligands by rapid photolysis,[2] and are thus important tools in the time-resolved crystallographic studies[3] of structural changes at the atomic level during an enzymatic reaction. Here we present 2-nitrobenzylarsonium compounds **1**–**3** (Scheme 1) as photolabile precursors of cholinergic ligands



Scheme 1. Synthesis of **1**–**3**.

that contain arsenic as a heavy-atom. They were prepared to facilitate an investigation of the catalytic process of cholinesterases by time-resolved crystallography.

Both acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) hydrolyze the neurotransmitter acetylcholine very rapidly to acetate and choline.[4] Although the three-dimensional structures of AChE[5] and of several AChE-inhibitor complexes[6] have been solved, questions concerning the traffic of substrate and products to and from the active

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site, raised in view of the high turnover rate ($20\,000\ s^{-1}$), remain unanswered.[7] An ideal approach to investigating these issues is the emergent time-resolved Laue crystallography[3] using caged cholinergic ligands.[8–11] These ligands are able to photorelease either choline (enzyme product) or carbamylcholine (enzyme substrate), allowing the initiation and control of the catalytic process of AChE at different stages.[11] The three-dimensional structure of the AChE complex obtained by the Laue method with a data collection time of 1 ms[12] is comparable to that obtained by conventional monochromatic crystallography. However, it can be difficult to locate choline in the electron density maps during time-resolved crystallographic studies, because the enzyme active site is filled with a number of water molecules[5, 6] whose electron density signals would, very likely, blend with those of choline. The use of heavy-atom derivatives of choline, such as arsenocholine, should help to overcome this problem since they should display stronger peaks in the electron density maps. Thus, arsenic has been incorporated as a heavy-atom substitute for nitrogen in the choline moiety of the caged cholinergic ligands **1**–**3**. Probes **1** and **2** are potential photolabile precursors of arsenocholine for the time-resolved crystallographic studies on cholinesterases, and probe **3** is one of arsenocarbamylcholine, provided they retain the required biochemical and photochemical properties. Here we report their syntheses, photochemical and biochemical characterizations, as well as the X-ray structures of **2** and its N-homologue.

The arsonium compounds **1**–**3** (Scheme 1) were synthesized almost quantitatively by substitution of the corresponding iodo precursors with trimethylarsane. The X-ray structure of **2** (Figure 1a) shows that the geometry about the arsenic atom is perfectly tetrahedral, similar to that found in arsenobetaine[13] and acetylarsenocholine.[14] Furthermore, the 2-nitrobenzyl and choline moieties of **2** are isostructural with those of its N-homologue (Figure 1b). As expected, the arsenic atom in **2** gave a much stronger diffraction signal than the corresponding nitrogen atom in the homologue (Figure 2).

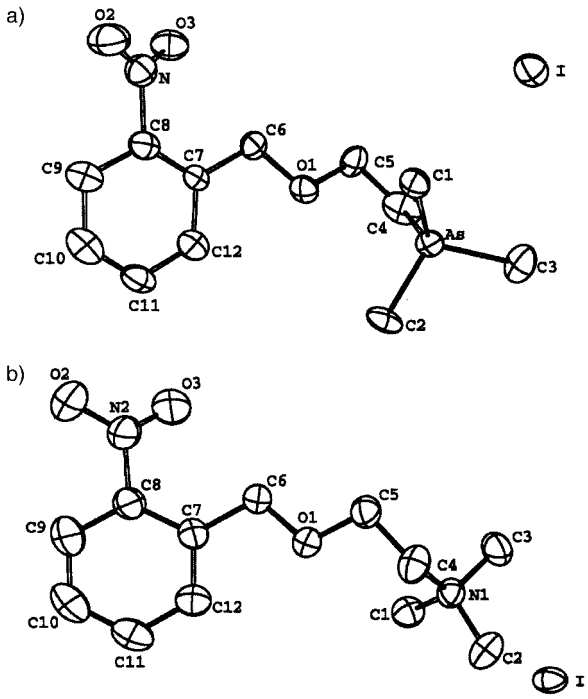

Figure 1. Crystal structures of **2** (a) and its N-homologue (b).

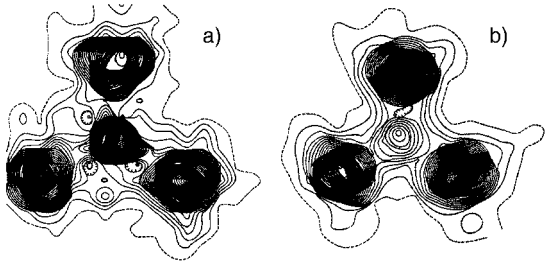

Figure 2. Electron density maps of the trimethylarsonium moiety (a) in **2** and the trimethylammonium moiety (b) in the N-homologue. The plane containing the three methyl groups was chosen for the calculation. The central spheroid in (a) is the As atom of **2**, while that in (b) is the corresponding N atom of the N-homologue.

The arsonium compounds **1**–**3** showed inhibitory properties on both purified *Torpedo* AChE and human serum BuChE (Table 1). The inhibition on BuChE is purely competitive, while that on AChE is of mixed competitive/noncompetitive type. The values of the inhibition constants (Table 1) are very similar to those obtained with the N-homologues,[8, 11] showing that quaternary arsonium salts are indeed satisfactory substitutes for ammonium salts with these cholinergic enzymes. These results support earlier findings,[15] where acetylarsenocholine was described as a cholinergic agonist for the nicotinic and muscarinic receptors as well as a substrate for AChE.

Table 1. Photofragmentation parameters and inhibition constants on cholinesterases of **1**–**3**.

Compd	k [s^{-1}][a]	Φ[b]	$K_{i(A)}$ [μM][c]	$K_{i(B)}$[μM][d]
1	9.1×10^4	0.26	17	18
2	2.2×10^2	0.17	4	11
3	5.1×10^4	0.33	63	29

[a] Rate constant of photofragmentation in 0.1M phosphate buffer, pH 6.45. [b] Quantum yield determined for 365 nm irradiation. [c] Inhibition constant on acetylcholinesterase (AChE). [d] Inhibition constant on butyrylcholinesterase (BuChE).

The use of **1**–**3** for time-resolved crystallographic studies depends critically on the rate and the quantum yield of their photofragmentation to give the desired products. The kinetics of the photochemical fragmentation were investigated by flash laser photolysis at 308 nm. The transient product, detected around 400 nm (Figure 3), is characteristic of an *aci*-nitro intermediate,[16] similar to what was observed for the N-homologue.[8, 9] The kinetics of photofragmentation (Figure 3) depend upon the nature of the released molecule, the substituent at the α-benzylic position (Table 1), and the pH value (data not shown); all of these parameters are in agreement with the currently favored fragmentation mechanism.[16] Most importantly, the half-times of the *aci*-nitro intermediates of **1** and **3** were around 10 μs, which is compatible with the enzyme turnover rate. Furthermore, the quantum yields (Table 1) of **1**–**3**, determined by comparing the extent of their photoconversion

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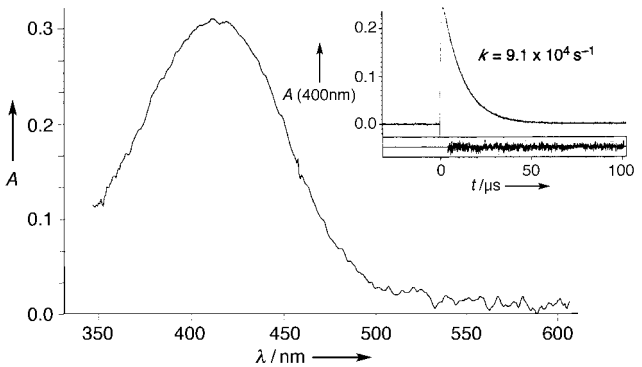

Figure 3. Spectrographic trace of the transient absorption (1 μs delay) after 308 nm laser flash photolysis of **1** (0.3 mM **1** in 0.1 mM phosphate buffer, pH 6.45, 20 °C). Insert: Kinetic trace of the transient absorbance changes at 400 nm.

at 365 nm with that of 1-(2-nitrophenyl)ethyl carbamylcholine ($\Phi = 0.29$),[9] are sufficient for the envisioned applications.

Thus, the arsonium compounds **1**–**3** are heavy-atom analogues of caged cholinergic ligands, and they possess biochemical and photochemical properties very similar to those of their N-homologues. The structural similarities between **2** and its N-homologue, and the strong X-ray diffracting effect of arsenic, confer to the As probes the desired properties for crystallographic studies on cholinesterases. Most importantly, **1** and **3** show excellent kinetic properties and quantum yields for the photorelease of arsenocholine and arsenocarbamylcholine, respectively. Thus they hold much promise in providing a decisive contribution to future time-resolved crystallographic studies on the catalytic mechanism of cholinesterases, and they might also be applicable to other systems.

Experimental Section

All the details for the photochemical reactions and the inhibition of ligands on cholinesterases can be found in references [8,10]. Crystallographic data (excluding structure factors) for the structures (**2** ($C_{12}H_{19}NO_3AsI$) and its *N*-homologue ($C_{12}H_{19}N_2O_3I$)) reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication nos CCDC-102120 and CCDC-102121, respectively. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

1: White powder; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.56$ (d, 3H, $J = 6.2$ Hz), 2.26 (s, 9H), 3.09–3.17 (m, 2H), 3.70–3.86 (m, 2H), 5.06 (q, 1H, $J =$ 6.3 Hz), 7.44–7.52 (m, 1H), 7.60–7.76 (m, 2H), 7.79 (dd, 1H, $J = 1.1$, 8.0 Hz); MS (positive-ion FAB): *m/z* (%): 313.8 (100) [$C_{13}H_{21}NO_3As$]; elemental analysis calcd for $C_{13}H_{21}NO_3AsI$: C 35.40, H 4.80, N 3.18, As 16.99; found: C 35.47, H 4.76, N 3.09, As 17.03.

2: White crystals; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 2.19$ (s, 9H), 3.22 (t, 2H, $J = 5.8$ Hz), 4.05 (t, 2H, $J = 5.8$ Hz), 4.90 (s, 2H), 7.52–7.69 (m, 3H), 8.00 (dd, 1H, $J = 1.3$, 7.8 Hz); MS (positive-ion FAB): *m/z* (%): 299.8 (100) [$C_{12}H_{19}NO_3As$].

3: Very hygroscopic white powders; ^{1}H NMR (200 MHz, CD_3CN): $\delta = 1.56$ (d, 3H, $J = 6.9$ Hz), 1.91 (s, 9H), 2.67 (t, 2H, $J = 5.8$ Hz), 4.33 (t, 2H, $J =$ 5.8 Hz), 5.10–5.21 (m, 1H), 6.77 (s br, 1H), 7.44–7.53 (m, 1H), 7.71–7.76 (m, 2H), 7.85 (d, 1H, $J = 8.8$ Hz); MS (positive-ion FAB): *m/z* (%): 356.8 (100) [$C_{14}H_{22}N_2O_4As$]; elemental analysis calcd for $C_{14}H_{22}N_2O_4AsI$: C 34.73, H 4.58, N 5.79, As 15.48; found: C 34.86, H 4.62, N 5.88, As 15.45.

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