A straightforward preparation of primary alkyl triflates and their utility in the synthesis of derivatives of ethidium

Steven A. Ross, Marguerite Pitié and Bernard Meunier *

Laboratoire de Chimie de Coordination du CNRS, 205 route de Narbonne, 31077 Toulouse Cedex 4, France

Received (in Cambridge, UK) 29th October 1999, Accepted 2nd December 1999

The reaction of primary alcohols with trifluoromethanesulfonic anhydride in the presence of poly(4-vinylpyridine) [or poly(2,6-di-*tert*-butyl-4-vinylpyridine)] allows the isolation of the corresponding alkyl triflates in good yields. The quaternisation of 3,8-bis(ethoxycarbonylamino)-6-phenylphenanthridine (**1**) using these alkyl triflates was found to proceed smoothly at room temperature in nitrobenzene or chlorobenzene. Deprotection of these quaternary compounds using concentrated hydrobromic acid was found to be a more useful procedure than the previously reported use of concentrated sulfuric acid. The synthesis of the novel ethidium derivative 3,8-diamino-5-(5-aminopentyl)-6-phenylphenanthridinium bromide (**12**) is reported.

Introduction

The binding of polyaromatic heterocyclic compounds to nucleic acids by insertion between adjacent base pairs (or "intercalation") was first proposed by Lerman in 1961.[1] This phenomenon is exhibited by a wide range of synthetic and naturally occurring derivatives, including acridines,[2] some antibiotics[3] and certain DNA groove-binding proteins.[4] 3,8-Diamino-5-ethyl-6-phenylphenanthridinium (or "ethidium") bromide was one of the first intercalators to be studied.[5] This compound exhibits anti-trypanosomal activity and is also widely used as a nucleic acid staining agent, due to the significant fluorescence enhancement which it exhibits upon intercalation.

Since the first early reports on the synthesis of ethidium bromide,[6] there has been a general lack of data on the preparation of its derivatives. This perhaps reflects some of the synthetic difficulties involved with this class of compound.

Hertzberg and Dervan[7] have reported the synthesis of an Fe(II)–EDTA derivative of ethidium (MPE) where functionalization was achieved through a carboxylate group at the *para* position of the 6-phenyl ring. MPE is used as a footprinting agent, through its ability to generate hydroxyl radicals in the close vicinity of DNA *via* a Fenton-type reaction.[8] Several other derivatives have been reported where the exocyclic amino groups of ethidium have been targeted as the starting points for further functionalization.[9] It should be stressed, however, that the attachment of a novel group to an ethidium derivative will have an effect upon the DNA binding orientation and, in particular, the orientation of the attached group with respect to DNA. Such considerations will be very important if the attached group is expected to have preferred reactivity in the major or minor grooves.

We were interested in developing a strategy for preparing derivatives where the point of attachment was *via* the quaternary nitrogen atom, partly since there has only been one derivative of this type previously reported,[10] and we were interested in the synthetic opportunities which would be made available by such an approach.

Results and discussion

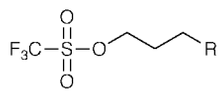
Preparation of alkyl triflates

Our initial approach was to prepare the bis(*tert*-butoxycarbonylamino) (BOC) derivative of 3,8-diamino-6-phenylphenanthridine. However, the synthesis of this material from the parent aromatic diamine and di-*tert*-butyl dicarbonate was very low yielding after chromatographic separation. Several authors have reported synthetic difficulties in the preparation of BOC derivatives of aromatic amines due to side reactions, such as bis-carbamoylation, urea formation and decomposition to isocyanates.[11] We therefore reverted to the previously reported high-yielding synthesis of 3,8-bis(ethoxycarbonylamino)-6-phenylphenathridine (**1**).[6] Quaternisation of this compound has been reported using tosylate esters in nitrobenzene at 155 °C. We wished to pursue the alkylation of this compound under less harsh conditions and so we investigated the reaction of trifluoromethanesulfonic acid esters under ambient conditions.

Trifluoromethanesulfonic acid esters are usually prepared *via* the reaction of the corresponding alcohol with triflic anhydride at low temperature in the presence of a non-nucleophilic base. The extreme electrophilicity of alkyl triflates can preclude the use of pyridine as the base in their preparation, due to alkylation of pyridine.[12] This has necessitated the use of hindered bases (such as 2,6-di-*tert*-butyl-4-methylpyridine) in some instances. During the reaction of ethyl 6-hydroxyhexanoate with triflic anhydride in the presence of 2,6-di-*tert*-butyl-4-methylpyridine, we found that our product was contaminated with the hindered base. Since alkyl triflates are not particularly stable, we prepared the esters prior to use and purified them by column chromatagraphy each time. Poly(2,6-di-*tert*-butyl-4-vinylpyridine) thus appeared a logical choice of base to use in this reaction, due to its ease of removal as the triflate salt by filtration. Indeed, reaction of ethyl 6-hydroxyhexanoate with 1 equivalent of triflic anhydride in the presence of 2 equivalents of hindered polymer base in dichloromethane at room temperature afforded the alkyl triflate in 91% yield after filtration, washing with bicarbonate solution and evaporation of solvent.

The same reaction can be carried out using poly(vinylpyridine) as the polymer-bound base, which also affords the alkyl triflates in yields of around 90%. The much lower cost of poly(vinylpyridine) makes this a more interesting synthetic tool. Alkylation of vinylpyridine using primary alkyl triflates has been reported previously to be extremely rapid at room temperature.[13] However, it would appear that alkylation of poly(vinylpyridine) is sufficiently slow to be a negligible side-reaction under our conditions.

Alkyl triflates of eight different primary alcohols were prepared to test the general utility of this reaction. These comprised *N*-*tert*-butoxycarbonyl-6-aminohexanol, *N*-*tert*-butoxycarbonyl-4-aminobutanol, *N*-ethoxycarbonyl-6-aminohexanol, butanol (alkyl triflate **2**), ethyl 6-hydroxyhexanoate (ester **3**), *N*-(5-hydroxypentyl)phthalimide (ester **4**), 5-(trifluoroacetamido)pentanol (ester **5**) and 3-(*tert*-butyldimethylsilyloxy)-propanol (ester **6**). Five of these eight alcohols **2**–**6** gave clean



2 R = CH_3

3 R = $CH_2CH_2C(O)OCH_2CH_3$

4 R = CH_2CH_2(Pth) Pth = *N*-Phthalimide

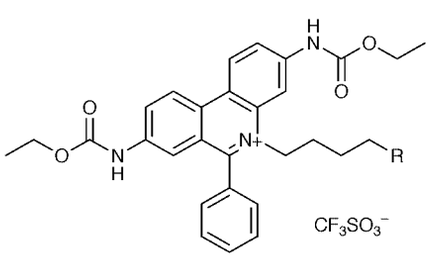
5 R = $CH_2CH_2NHC(O)CF_3$

6 R = $OSi(CH_3)_2C(CH_3)_3$

conversion to the alkyl triflates (as monitored by ^{1}H NMR) without any need to purify the product. The only exceptions were the carbamate derivatives, which showed up to 30% of impurities by NMR. These impurities are probably due to acid catalysed hydrolysis of the carbamate groups. Alternatively, the carbamate N atom may be sufficiently nucleophilic to undergo an intramolecular reaction with the alkyl triflate. For example, Nicolaou and co-workers[14] have reported the nucleophilic displacement of primary alkyl triflates by the nitrogen atom of the trifluoroacetamido group.

Alkylation reactions

Initial attempts to react alkyl triflates **2**–**5** with **1** at elevated temperatures gave a complex mixture of products. However, the reactions were found to proceed smoothly with yields of around 60% at room temperature in nitrobenzene or chlorobenzene, to give the quaternary triflate salts **7**–**10**. These mild conditions



7 R = H

8 R = $CH_2C(O)OCH_2CH_3$

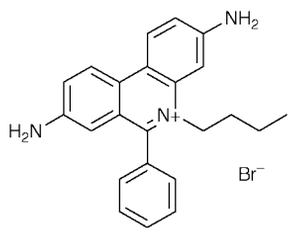
9 R = CH_2(Pth) Pth = *N*-phthalimide

10 R = $CH_2NHC(O)CF_3$

contrast with the previously reported reaction of alkyl tosylates at 155 °C.[6] Protonated **1**H^+ $CF_3SO_3^-$ was found to be the other component in the reaction mixture, presumably formed by elimination of triflic acid from the esters. No other products were observed.

Deprotection studies

The aromatic ethyl carbamate protecting groups in this class of compounds are normally removed by heating in conc. sulfuric acid at 120 °C for 2 hours, followed by neutralisation and precipitation.[6] However, we found that isolation of our products from conc. sulfuric acid was problematic and thus we tried a range of acids as alternatives. No deprotection was observed with trifluoroacetic acid, either at room temperature or at reflux, nor with concentrated hydrochloric acid at reflux. Deprotection was observed with concentrated hydrobromic acid at reflux overnight. This has the advantage that the acid is easily removed under reduced pressure, unlike conc. sulfuric acid. Reaction of butyl quaternary derivative **7** with conc. HBr, followed by removal of solvent and purification by chromatography afforded ethidium analogue **11** in 90% yield, which was

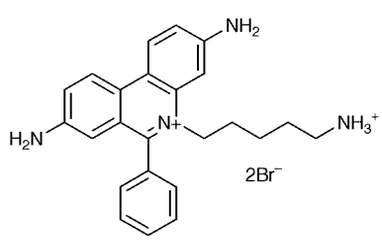


11

confirmed by comparison of the ^{1}H NMR sample with a commercial sample of ethidium bromide.

However, on several occasions an unusual impurity was observed in large excess, which exhibited a triplet with a coupling constant of 51 Hz in the ^{1}H NMR spectrum. This was attributed to the decomposition of the triflate anion to give difluoromethanesulfonate, which is consistent with the ^{19}F–^{1}H geminal coupling constant observed. Repeated chromatography could not remove this product. This problem was overcome by exchanging the counter-ion to bromide prior to reaction with concentrated HBr.

Our interests lay in the preparation of an ethidium derivative where further functionalisation could be readily achieved *via* the presence of an appropriate group on the alkyl chain attached to the quaternary nitrogen. A primary amine was chosen, since this would be a much better nucleophile than the aromatic amines at the 3 and 8 positions, which are known to be very poor nucleophiles (reaction of ethidium bromide with glutaric anhydride gives incomplete substitution after 48 h at 70 °C in DMF).[9] Reaction of the bromide salt of **10** with conc. HBr at reflux for 12 hours, followed by removal of solvent and purification by chromatography gave the expected product **12** as



12

a hygroscopic solid, which represents a useful starting material for the synthesis of ethidium derivatives.

Experimental

^{1}H NMR spectra were recorded on a Bruker AC 250 instrument running at 250.134 MHz. Methylene groups adjacent to the quaternary phenanthridinium centre are labelled as $N_{quat}CH_2$ for assignment.

All chemicals were used as supplied unless stated. Trifluoromethanesulfonic anhydride was freshly prepared prior to use by distillation of a 1 : 1 w/w mixture of trifluoromethanesulfonic acid and phosphorus pentoxide.[15] *N*-*tert*-Butoxycarbonyl-6-aminohexanol,[16] *N*-*tert*-butoxycarbonyl-4-aminobutanol,[16] 5-(trifluoroacetamido)pentanol,[17] *N*-(5-hydroxypentyl)phthalimide[18] and 3-(*tert*-butyldimethylsilyloxy)propanol[19] were prepared according to literature methods. **CAUTION**: ethidium

and its derivatives are believed to be carcinogenic; appropriate care should be taken when dealing with these products.

3,8-Bis(ethoxycarbonylamino)-6-phenylphenanthridine (1)

This was prepared according to the method described by Watkins.[6] The ^{1}H NMR data is included here for completeness, since this has not been previously reported. ^{1}H NMR (DMSO-d_6): 1.33 (3H, t, J = 7 Hz, CH_3), 1.40 (3H, t, J = 7 Hz, CH_3), 4.23 (2H, q, J = 7 Hz, CH_2), 4.30 (2H, q, J = 7 Hz, CH_2), 7.68 (3H, m, ArH), 7.80 (2H, m, ArH), 7.95 (1H, dd, J = 9, 1 Hz, ArH), 8.09 (1H, dd, J = 9, 1 Hz, ArH), 8.32 (1H, d, J = 1 Hz, ArH), 8.43 (1H, d, J = 1 Hz, ArH), 8.75 (1H, d, J = 9 Hz, ArH), 8.84 (1H, d, J = 9 Hz, ArH), 10.12 (1H, s, NH) and 10.15 ppm (1H, s, NH); FAB-MS: 429 (M^+, 100%).

Preparation of alkyl triflates

The following general procedure was employed. Poly(vinylpyridine) (220 mg, 2 mmol) was added to dichloromethane (5 mL), followed by trifluoromethanesulfonic anhydride (282 mg, 1 mmol). The primary alcohol (0.95 mmol) was then added (as a solution in dichloromethane) in a dropwise fashion over 1 minute at room temperature. The reaction mixture was stirred for 5 minutes, then filtered under gravity. The poly(vinylpyridinium triflate) precipitate was washed with 2 mL of dichloromethane. The combined organics were washed once with saturated $NaHCO_3$ solution, dried ($MgSO_4$) and concentrated under vacuum to give the products as colourless oils, which gave a pinkish hue upon standing. Dichloromethane solutions of the products became black over time. The methylene group adjacent to the alkyl triflate gives a characteristic triplet at approximately 4.5 ppm in the ^{1}H NMR spectrum, compared to the starting alcohols, where the corresponding methylene group is observed at approximately 3.5 ppm.

Butyl triflate (2). Yield 189 mg, 92%; ^{1}H NMR (CD_2Cl_2): 0.78 (3H, t, J = 7 Hz), 1.34 (2H, m), 1.88 (2H, m) and 4.49 ppm (2H, t, J = 6 Hz).

Ethyl 6-(trifluoromethylsulfonyloxy)hexanoate (3). Yield 265 mg, 91%; ^{1}H NMR (CD_2Cl_2): 1.23 (3H, t, J = 7 Hz), 1.45 (2H, m), 1.66 (2H, m), 1.82 (2H, m), 2.32 (2H, t, J = 7 Hz), 4.10 (2H, q, J = 7 Hz) and 4.56 ppm (2H, t, J = 6 Hz).

5-Phthalimidopentyl triflate (4). Yield 317 mg, 87%; ^{1}H NMR (CD_2Cl_2): 1.46 (2H, m), 1.70 (2H, m), 1.89 (2H, m), 3.68 (2H, t, J = 7 Hz), 4.56 (2H, t, J = 6 Hz) and 7.76 ppm (4H, m).

5-(Trifluoroacetamido)pentyl triflate (5). Yield 256 mg, 81%; ^{1}H NMR ($CDCl_3$): 1.38 (2H, m), 1.55 (2H, m), 1.77 (2H, m), 3.23 (2H, q, J = 7 Hz), 4.46 (2H, t, J = 6 Hz) and 8.01 ppm (1H, br s).

3-(*tert*-Butyldimethylsilyloxy)propyl triflate (6). Yield 294 mg, 84%; ^{1}H NMR (CD_2Cl_2): 0.07 (6H, s), 0.89 (9H, s), 1.92 (2H, m), 3.74 (2H, t, J = 6 Hz) and 4.53 ppm (2H, t, J = 6 Hz).

Alkylation of 3,8-bis(ethoxycarbonylamino)-6-phenylphenanthridine

The following general procedure was employed. The appropriate alkyl triflate (1 mmol) was added to a solution of **1** (1 mmol) in nitrobenzene (5 mL) and stirred at room temperature for 18 hours. The nitrobenzene was then removed by column chromatography using 100% dichloromethane. The product was then eluted from the column using a dichloromethane–methanol mixture (9 : 1). Evaporation of solvent afforded the pure product as a bright yellow fluorescent solid.

5-Butyl-3,8-bis(ethoxycarbonylamino)-6-phenylphenanthridinium trifluoromethanesulfonate (7). Yield 387 mg, 61%; ^{1}H NMR (CD_2Cl_2): 0.81 (3H, t, J = 7 Hz, CH_3), 1.24 (3H, t, J = 7 Hz, CH_3), 1.35 (3H, t, J = 7 Hz, CH_3), 1.37 (2H, m, CH_2), 1.94 (2H, br m, CH_2), 4.13 (2H, q, J = 7 Hz, CH_2OCO), 4.27 (2H, q, J = 7 Hz, CH_2OCO), 4.72 (2H, br t, J = 8 Hz, $N_{quat}CH_2$), 7.48 (1H, d, J = 2 Hz, ArH), 7.51 (1H, d, J = 2 Hz, ArH), 7.77–7.88 (5H, m, ArH), 8.19 (1H, dd, J = 9, 2 Hz, ArH), 8.25 (1H, dd, J = 9, 2 Hz, ArH), 8.58 (1H, d, J = 9 Hz, ArH), 8.65 (1H, d, J = 9 Hz), 8.74 (1H, s, NH) and 8.92 ppm (1H, s, NH); FAB-MS 486 (M^+ cation, 100%) (Found: C, 51.9; H, 4.7; N, 5.7. $C_{30}H_{32}F_3N_3O_7S{\cdot}CH_2Cl_2$ requires: C, 51.7; H, 4.7; N, 5.8%).

3,8-Bis(ethoxycarbonylamino)-5-(5-ethoxycarbonylpentyl)-6-phenylphenanthridinium trifluoromethanesulfonate (8). Yield 454 mg, 63%; ^{1}H NMR (DMSO-d_6): 1.31 (3H, t, J = 7 Hz, CH_3), 1.35 (3H, t, J = 7 Hz, CH_3), 1.42 (3H, t, J = 7 Hz, CH_3), 1.3–1.5 (4H, m, CH_2CH_2), 2.08 (2H, br m, CH_2), 2.32 (2H, t, J = 6 Hz, CH_2COOEt), 4.14 (2H, q, J = 7 Hz, CH_2OCO), 4.19 (2H, q, J = 7 Hz, CH_2OCO), 4.34 (2H, q, J = 7 Hz, CH_2OCO), 4.64 (2H, br t, J = 8 Hz, $N_{quat}CH_2$), 7.8–8.0 (6H, m, ArH), 8.22 (1H, dd, J = 9, 2 Hz, ArH), 8.34 (1H, dd, J = 9, 2 Hz, ArH), 8.78 (1H, s, ArH), 9.12 (1H, d, J = 9 Hz, ArH), 9.19 (1H, d, J = 9 Hz, ArH), 10.39 (1H, s, NH) and 10.67 ppm (1H, s, NH); FAB-MS 572 (M^+ cation, 100%) (Found: C, 47.1; H, 4.1; N, 4.8. $C_{34}H_{38}F_3N_3O_9S{\cdot}\frac{3}{2}CH_2Cl_2$ requires: C, 47.1; H, 4.7; N, 4.7%).

3,8-Bis(ethoxycarbonylamino)-6-phenyl-5-(5-phthalimidopentyl)phenanthridinium trifluoromethanesulfonate (9). Yield 452 mg, 57%; ^{1}H NMR (DMSO-d_6): 1.31 (3H, t, J = 7 Hz, CH_3), 1.35 (3H, t, J = 7 Hz, CH_3), 1.4 (2H, m, CH_2), 1.56 (2H, br m, CH_2), 2.09 (2H, br m, CH_2), 3.61 (2H, t, J = 6 Hz, CH_2N-[phthalimide]), 4.18 (2H, q, J = 7 Hz, CH_2OCO), 4.27 (2H, q, J = 7 Hz, CH_2OCO), 4.64 (2H, br t, J = 8 Hz, $N_{quat}CH_2$), 7.87 (5H, s, ArH), 7.91 (1H, d, J = 2 Hz, ArH), 7.89 (4H, s, ArH-[phthalimide]), 8.24 (1H, dd, J = 9, 2 Hz, ArH), 8.36 (1H, dd, J = 9, 2 Hz, ArH), 8.76 (1H, s, ArH), 9.13 (1H, d, J = 9 Hz, ArH), 9.19 (1H, d, J = 9 Hz, ArH), 10.39 (1H, s, NH) and 10.62 ppm (1H, s, NH); FAB-MS 645 (M^+ cation, 100%) (Found: C, 58.8; H, 4.6; N, 6.7. $C_{39}H_{37}F_3N_4O_9S$ requires: C, 58.9; H, 4.6; N, 7.0%).

3,8-Bis(ethoxycarbonylamino)-6-phenyl-5-(5-trifluoroacetamidopentyl)phenanthridinium trifluoromethanesulfonate (10). Yield 372 mg, 49%; ^{1}H NMR (DMSO-d_6): 1.31 (3H, t, J = 7 Hz, CH_3), 1.42 (3H, t, J = 7 Hz, CH_3), 1.3–1.5 (4H, m, CH_2-CH_2), 2.09 (2H, br m, CH_2), 3.23 (2H, m, $CH_2NHCOCF_3$), 4.19 (2H, q, J = 7 Hz, CH_2OCO), 4.36 (2H, q, J = 7 Hz, CH_2OCO), 4.65 (2H, br t, J = 8 Hz, $N_{quat}CH_2$), 7.8–8.0 (6H, m, ArH), 8.22 (1H, dd, J = 9, 2 Hz, ArH), 8.37 (1H, dd, J = 9, 2 Hz, ArH), 8.79 (1H, s, ArH), 9.13 (1H, d, J = 9 Hz, ArH), 9.18 (1H, d, J = 9 Hz, ArH), 9.48 (1H, br t, J = 6 Hz, $NHCOCF_3$), 10.39 (1H, s, NHCOOEt) and 10.69 ppm (1H, s, NHCOOEt); FAB-MS 611 (M^+ cation, 100%) (Found: C, 49.6; H, 3.9; N, 7.0. $C_{33}H_{34}F_6N_4O_8S{\cdot}\frac{1}{2}CH_2Cl_2$ requires: C, 50.1; H, 4.3; N, 7.0%).

Deprotection reactions

The following procedure was employed to prepare products **11** and **12**. The alkylated triflate salt **7** (or **10**) (1 mmol) was dissolved in dichloromethane (50 mL), then washed with dilute hydrobromic acid (1 M, 4 × 100 mL). The organic layer was separated and concentrated under vacuum (addition of $MgSO_4$ as drying agent should be avoided, as this precipitates the bromide salt from dichloromethane). Hydrobromic acid (10 mL, 48%) was then added and the solution was heated at reflux for 18 hours. The acid was removed under vacuum and the crude residue was purified by column chromatography (dichloromethane–methanol, 4 : 1) to give the products as intensely coloured red solids. The aromatic amine groups were found to be unprotonated after this work-up procedure, being clearly observed in the ^{1}H NMR spectra at approx. 6.1 and 6.6 ppm

with the correct integration, and in agreement with the spectrum of commercially available ethidium bromide.

3,8-Diamino-5-butyl-6-phenylphenanthridinium bromide (11). Yield 395 mg, 91%; ^{1}H NMR (DMSO-d_6): 0.78 (3H, t, J = 7 Hz, CH_3), 1.32 (2H, br m, CH_2), 1.93 (2H, br m, CH_2), 4.46 (2H, br m, $N_{quat}CH_2$), 6.08 (2H, br s, $ArNH_2$), 6.37 (1H, s, ArH), 6.57 (2H, br s, $ArNH_2$), 7.44 (1H, d, J = 9Hz, ArH), 7.47 (1H, s, ArH), 7.64 (1H, d, J = 9Hz, ArH), 7.81 (5H, m, ArH), 8.71 (2H, d, J = 9Hz, ArH) and 8.76 ppm (2H, d, J = 9 Hz, ArH); ES-MS 342 (M^+ cation, 100%) (Found: C, 60.1; H, 6.3; N, 8.8. $C_{23}H_{24}BrN_3 \cdot 2H_2O$ requires: C, 60.3; H, 6.1; N, 9.2%).

3,8-Diamino-5-(5-aminopentyl)-6-phenylphenanthridinium bromide (hydrobromide salt) (12). Yield 374 mg, 83%; ^{1}H NMR (DMSO-d_6): 1.35 (2H, m, CH_2), 1.54 (2H, m, CH_2), 1.96 (2H, br m, CH_2), 2.80 (2H, t, J = 10 Hz, $CH_2NH_3^+$), 4.42 (2H, br m, $N_{quat}CH_2$), 6.09 (2H, s, $ArNH_2$), 6.35 (1H, d, J = 2 Hz, ArH), 6.66 (2H, s, $ArNH_2$), 7.47 (1H, d, J = 9 Hz, ArH), 7.64 (1H, s, ArH), 7.68 (1H, d, J = 2 Hz, ArH), 7.89 (5H, m, ArH), 7.97 (3H, br s, $CH_2NH_3^+$), 8.73 (1H, d, J = 9 Hz) and 8.77 ppm (1H, d, J = 9 Hz); ES-MS 371 (M^+ cation, 100%).

Acknowledgements

We thank the European Commission for a Marie Curie Fellowship (Training and Mobility of Researchers; Grant No. ERBFMBICT972447) to S. A. R.

References

1 L. S. Lerman, *J. Mol. Biol.*, 1961, **3**, 18.
2 A. Albert, *The Acridines*, 2nd edn., ed. E. Arnold, London, 1966; S. A. Ross, M. Pitié and B. Meunier, *Eur. J. Inorg. Chem.*, 1999, 557; E. M. Nelson, K. M. Tewey and L. F. Liu, *Proc. Natl. Acad. Sci. USA*, 1984, **81**, 1361.
3 W. Priebe, *Anthracycline Antibiotics: New Analogues, Methods of Delivery and Mechanisms of Action*, ACS Symposium Series 574, American Chemical Society, Washington DC, 1995; *Molecular Basis of Specificity in Nucleic Acid-Drug Interactions*, eds. B. Pullman and J. Jortner, Kluwer Academic Publishers, Dordrecht, 1990; I. H. Goldberg, *Free Radicals Biol. Med.*, 1987, **3**, 41; D. L. Boger and H. Cai, *Angew. Chem., Int. Ed.*, 1999, **38**, 448.
4 M. H. Werner, A. M. Gronenborn and G. M. Clore, *Science*, 1996, **271**, 778; J. L. Kim and S. K. Burley, *Nat. Struct. Biol.*, 1994, **1**, 638.
5 M. J. Waring, *J. Mol. Biol.*, 1965, **13**, 269; J. B. LePecq and C. Paoletti, *J. Mol. Biol.*, 1967, **27**, 87; R. J. Douthart, J. P. Burnett, F. W. Beasley and B. H. Frank, *Biochemistry*, 1973, **12**, 214; G. Marx, H. Zhou, D. E. Graves and N. Osheroff, *Biochemistry*, 1997, **36**, 15884.
6 L. P. Walls, *J. Chem. Soc.*, 1947, 67; T. I. Watkins, *J. Chem. Soc.*, 1952, 3059; T. I. Watkins, *J. Chem. Soc.*, 1958, 1443.
7 R. P. Hertzberg and P. B. Dervan, *J. Am. Chem. Soc.*, 1982, **104**, 313; R. P. Hertzberg and P. B. Dervan, *Biochemistry*, 1984, **23**, 3934.
8 C. Walling and S. Kato, *J. Am. Chem. Soc.*, 1971, **93**, 4275.
9 J. Loccufier and E. Schacht, *Tetrahedron*, 1989, **45**, 3385; S. O. Kelley, R. E. Holmlin, E. D. A. Stemp and J. K. Barton, *J. Am. Chem. Soc.*, 1997, **119**, 9861.
10 Previously reported *N*-alkylated ethidium derivative.
11 T. A. Kelly and D. W. McNeil, *Tetrahedron Lett.*, 1994, **35**, 9003; T. J. Thornton and M. Jarman, *Synthesis*, 1990, 295; M. C. Venuti, R. A. Stephenson, R. Alvarez, J. J. Bruno and A. M. Strosberg, *J. Med. Chem.*, 1988, **31**, 2136; H.-J. Knölker, T. Braxmeier and G. Schlectingen, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 2497.
12 M. G. Ambrose and R. W. Binkley, *J. Org. Chem.*, 1983, **48**, 674.
13 W. K. Fife, P. Ranganathan and M. Zeldin, *J. Org. Chem.*, 1990, **55**, 5610.
14 R. Hirschmann, K. C. Nicolaou, S. Pietranico, E. M. Leahy, J. Salvino, B. Arison, M. A. Cichy, P. G. Spoors, W. C. Shakespeare, P. A. Sprengeler, P. Hamley, A. B. Smith III, T. Reisine, K. Raynor, L. Maechler, C. Donaldson, W. Vale, R. M. Freidinger, M. R. Cascieri and C. D. Strader, *J. Am. Chem. Soc.*, 1993, **115**, 12550.
15 P. J. Stang and T. E. Dueber, *Org. Synth.*, 1987, **Coll. Vol. VI**, 757.
16 P. G. Mattingly, *Synthesis*, 1990, 336.
17 S. G. Lokhov, M. A. Podyminogin, D. S. Sergeev, V. N. Silnikov, I. V. Kutyavin, G. V. Shishkin and V. P. Zarytova, *Bioconjugate Chem.*, 1992, **3**, 414.
18 R. Hamilton, B. J. Walker and B. Walker, *Tetrahedron Lett.*, 1993, **34**, 2847.
19 J. A. Marshall, B. G. Shearer and S. L. Crooks, *J. Org. Chem.*, 1987, **52**, 1236.

Paper a908389h