Synthesis of some unsymmetrical 2,6-diphenylpyridines for conjugation with analogues of the metal binding unit of bleomycin

J. Chem. Research (S), 2000, 502–503
J. Chem. Research (M), 2000, 1221–1236

Lina K. Mehta, John Parrick* and Samina Sadiq

Chemistry Section, Institute for Physical and Environmental Sciences, Brunel University, Uxbridge, Middlesex UB8 3PH, UK

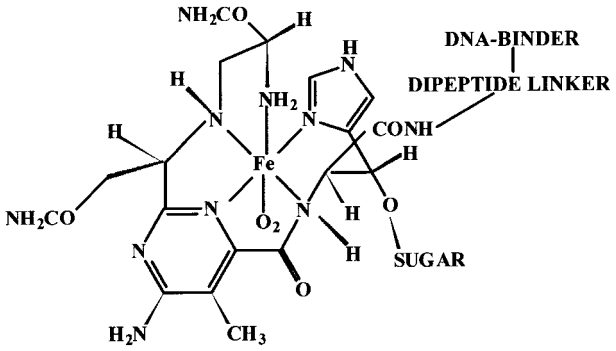
Some 2,6-diphenylpyridines, unsymmetrically substituted at the *para* position of the phenyl nuclei, have been prepared for incorporation into the molecular architecture of bleomycin analogues.

The clinically useful antitumour antibiotic bleomycin forms a complex **2** with iron and oxygen and its molecular architecture includes a metal complexing unit, a disaccharide, a dipeptide and a DNA binding unit which is a bithiazole derivative. The activated bleomycin-iron-oxygen complex **2** causes the cytotoxicity of the drug by degradation of DNA through strand scission at predominantly 5′-GC and 5′-GT sites. When the bithiazole nucleus is replaced by other units which bind to DNA, scission still occurs predominantly at those same sites, even though the preferences of the DNA binding units may be different.[1–6] This and other evidence[3,4,6] point to the conclusion that the bithiazole unit does not control sequence specificity but that it is controlled by the metal complexing domain.[7] More accessible analogues of this part of the molecule have a pyridine ring in place of the pyrimidine nucleus and have been shown to be cytotoxic in the presence of iron and oxygen but they have been found to be non-selective.[8–10,15–17]

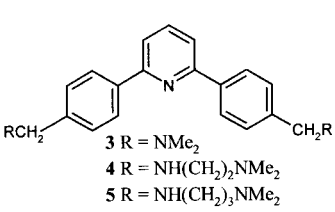
It has been shown that cytotoxicity is increased if certain compounds having little or no cytotoxicity are added to bleomycin. Such compounds are called ‘amplifiers’ and have two or more bonded but unfused rings.[22–26] It is believed that these amplifiers operate by expanding the minor groove so allowing easier access by the drug.[21]

The tricyclic, unsymmetrically substituted 2,6-diarylpyridines **3**–**5** are effective amplifiers of bleomycin cytotoxicity[25] and this paper reports the preparation of such compounds which were linked to pyridine based metal-complexing units **6**–**8**[10] to give novel bleomycin analogues whose bioreductive activation and cytotoxicity we have reported.[27]

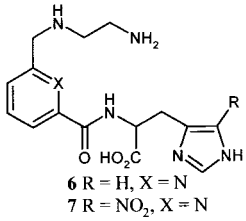
Few 2,6-diphenylpyridines with no other substituents on the heterocycle have been described. For the present purpose, it was necessary to prepare unsymmetrically substituted 2,6-diarylpyridines **9**, where one substituent terminates with a primary amino group (to facilitate the formation of a peptide link with the metal-binding domain **6**–**8**) and the other substituent terminates with an NMe_2 group (to become protonated and form an electrostatic link with DNA). There are few examples of unsymmetrically substituted 2,6-diarylpyridines in the literature.



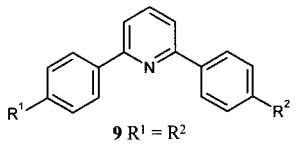
2



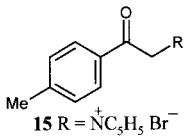
3 R = NMe_2
4 R = $NH(CH_2)_2NMe_2$
5 R = $NH(CH_2)_3NMe_2$



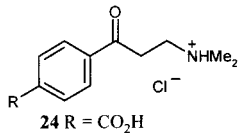
6 R = H, X = N
7 R = NO_2, X = N
8 R = H, X = N^+-O^-



9 $R^1 = R^2$

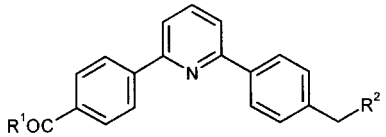


15 R = $\overset{+}{N}C_5H_5\ Br^-$

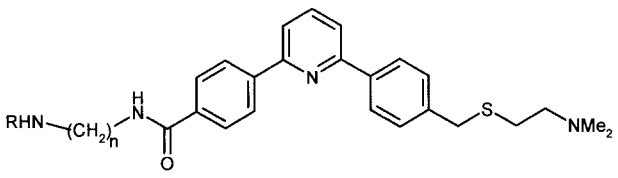


24 R = CO_2H

Several unsuccessful attempts were made to prepare suitable compounds **9** but these were eventually obtained using the Krohnke approach to pyridines.[33–35] The reaction of the pyridinium salt **15** and **24** in the presence of ammonium acetate gave the acid **28** which was converted in good yield



28 R^1 = OH, R^2 = H
29 R^1 = $NH(CH_2)_2NHBoc$, R^2 = H
30 R^1 = OEt, R^2 = H
31 R^1 = OEt, R^2 = Br
32 R^1 = OEt, R^2 = $S(CH_2)_2NMe_2$



33 R = H, n = 2
34 R = H, n = 4
35 R = H, n = 6
36 R = Ac, n = 2
37 R = Ac, n = 4
38 R = Ac, n = 6

* To receive any correspondence.

into the amide **29** by reaction with 2-(*tert*-butoxy-carbonylamino)ethylamine in the presence of 1-[3-(dimethylamino)propyl]-3-ethyl-carbodiimide hydrochloride and 1-hydroxybenzotriazole. However, attempted bromination by *N*-bromosuccinimide of the methyl group, which had proceeded smoothly in the structurally related cases, failed when applied to **29**.

The alternative approach of bromination of the simple ester **30,** obtained from the acid **28** proceeded smoothly to give **31** and led to a route to the desired compounds. Thus, replacement of the halogen by reaction with 2-(dimethylamino)ethane thiol gave the sulfide **32**. The ester group of **32** was directly converted into the amides **33**–**35** by treatment with an excess of the appropriate α,ω-diaminoalkane. The yields decreased as the chain lengths increased but were acceptable for the present purpose. It was intended to use the primary amino group in the formation of an amide bond with the ester group of the available[27] analogues of the metal binding domain of bleomycin. As a model for the DNA binding abilities of these bleomycin analogue-diarylpyridine conjugates, the primary amines were acetylated to give **36**–**38**.

Ethidium bromide binds with DNA through intercalation and this causes a large fluorescence enhancement due to the dye being surrounded by the hydrophobic region of DNA and hence the exclusion of water. The binding of other compounds to DNA can be assayed in a competitive experiment in which that compound is added to the ethidium bromide–DNA complex and the expulsion of the fluorophore from the complex is shown by a decrease in the fluorescence intensity.[36,37] The binding constant for bleomycin[38] with DNA is about 10^5 M^{-1} but the diarylpyridine amines **33**–**35** and the diarylpyridine amide with the longest chain **38** had a binding constant about an order of magnitude greater than bleomycin (Table 1). However, the diarylpyridine amides **36** and **37** had smaller binding constants, approximately three or four greater than that of bleomycin. As the chain lengths increased from two to six carbon atoms between the two amide nitrogen atoms in **36**–**38** so the binding constants increased from 3.4×10^5 to 1.1×10^6 M^{-1}.

We thank the EPSRC National Mass Spectrometry Service Centre, Swansea, for the accurate mass spectrometry data.

Techniques used: 1H NMR, IR and fluorescence spectroscopy, mass spectrometry, elemental analysis.

References: 38

Formulae: 38

Tables: 5

Received 18 June 2000; accepted 5 September 2000
Paper 00/395

References cited in this synopsis

1 N. Hamamichi, A. Natrajan and S.M. Hecht, *J. Am. Chem. Soc.*, 1992, **114**, 6278.
2 R.E. Kiluskie, H. Suguna, B. Yellin, N. Murugesan and S. M. Hecht, *J. Am. Chem. Soc.*, 1985, **107**, 260.
3 B.J. Carter, V.S. Murty, K.S. Reddy, S. Wang and S.M. Hecht, *J. Biol. Chem.*, 1990, **265**, 4193.
4 S.A. Kane, A. Natrajan and S.M. Hecht, *J. Biol. Chem.*, 1994, **269,** 10899.
5 J.C. Quada, Jr., M.J. Levy, and S.M. Hecht, *J. Am. Chem. Soc.*, 1993, **115**, 12171.
6 L. Huang, J.C. Quada and L.W. Lown, *Curr. Med. Chem.*, 1995, **2**, 543.
7 D. Suh and L.F. Povirk, *Biochemistry*, 1997, **36**, 4248.
8 J.P. Henichart, J.L. Bernier, R. Houssin, M. Lohez, A. Kenani and J.P. Catteau, *Biochem. Biophys. Res. Comm.*, 1985, **126**, 1036.
9 K. Shinozuka, H. Morishita, T. Yamazuki, Y. Sagiura, and H. Sawai, *Tetrahedron Lett.*, 1991, **32**, 6869.
10 J.A. Highfield, L.K. Mehta, J. Parrick, L.P. Candeias, and P. Wardman, *J. Chem. Soc., Perkin Trans. 1*,1999, 2343.
15 A. Kittaka, M. Otsuka, T. Suzuki, J. Kuwahara, Y. Sugiura, H. Umezawa and M. Ohno, *Tetrahedron Lett.*, 1986, **27**, 3639.
16 A. Kittaka, Y. Sugano, M. Otsuka and M. Ohno, *Tetrahedron*, 1988, **44**, 2821.
17 A. Kenani, M. Lohez, R. Houssin, N. Helbecque, J.L. Bernier, P. Lemay and J.P. Henichart, *Anti-Cancer Drug Design*, 1987, **2**, 47.
22 L. Huang, J.C. Quada, Jr. and J.W. Lown, *Bioconjugate Chem.*, 1995, **6**, 21.
23 L. Strekowski, W.D. Wilson, J. L. Mokrosz, M.J. Mokrosz, D.B. Harden, F.A. Tanious, R.L. Wydra and S.A. Crow, Jr., *J. Med. Chem.*, 1991, **34**, 580.
24 W.D. Wilson, L. Ratmeyer, M. Zhao, L. Strekowski and D. Boykin, *Biochemistry*, 1993, **32**, 4098.
25 L. Strekowski, A. Strekowska, R.A. Watson, F.A. Tanious, L.T. Nguyen and W. D. Wilson, *J. Med. Chem.*, 1987, **30**, 1415.
26 L. Strekowski, J.L. Mokrosz, F.A. Tanious, R.A. Watson, D. Harden, M. Mokrosz, W.D. Edwards and W.D. Wilson, *J. Med. Chem.*, 1988, **31**, 1231.
27 J. A. Highfield, L. K. Mehta, J. Parrick and P. Wardman, *Bioorg. Med. Chem.*, in press.
33 W. Zecher and F. Krohnke, *Chem. Ber.*, 1961, **94**, 690.
34 F. Krohnke and W. Zecher, *Angew. Chem.*, 1962, **74**, 811.
35 R. W. Rehberg and F. Krohnke, *Justus Liebigs Ann. Chem.*, 1968, **717**, 91.
36 J. B. LePecq and C. Paoletti, *J. Mol. Biol.*, 1967, **27**, 87.
37 A.R. Morgan, J.S. Lee, D.E. Pulleyblank, N.L. Murray and D.H. Evans, *Nucleic Acids Res.*, 1979, **7**, 547.
38 M. Chein, A.P. Grollma, and S.B. Horwitz, *Biochemistry*, 1977, **16**, 3641.