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SYNTHESIS OF AN IMIDAZO[1,2-e]PURINE-ACRIDINE HETERODIMER FOR TARGETING ABASIC SITES IN DNA

Philippe Belmont, Karine Alarcon, Martine Demeunynck,* Jean Lhomme

LEDSS, UMR CNRS 5616/Université J. Fourier, BP 53, 38041 Grenoble cédex 9, France

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Abstract: Cyclization of 8-bromo-9-alkylaminoethyl-adenine quantitatively affords a substituted imidazo[1,2-e]purine. The corresponding heterodimer, imidazo[1,2-e]purine-acridine, was prepared and its interaction with abasic site containing oligonucleotides was studied.

Abasic sites (or AP-sites) in DNA are potential targets in the design of new chemotherapeutic agents.[1,2] Abasic sites result from the loss of a nucleic base (purine or pyrimidine) and are produced in significantly higher rates during anticancer chemotherapy by a number of alkylating drugs. If not repaired, abasic sites may promote mutagenesis and cell-death. Their repair is thus a critical cellular activity, and two classes of enzymes, i. e. AP-endonucleases I and II, are involved in the key-step of the process. Molecule, such as bis-naphtalimide DMP-840, that is thought to interfere with the repair process is currently in phase I clinical trials.[3]

In preceding papers,[4-6] we reported the synthesis and biological studies of heterodimeric molecules in which a purine, adenine or 2,6-diaminopurine, is linked to a 9-aminoacridine nucleus by a polyamino linker. These molecules specifically recognize abasic sites in DNA and cleave the DNA strand through a β-elimination mechanism. The most active molecules, such as **1** (Fig. 1), cleave pBR322 plasmid containing 2 abasic sites at nanomolar concentration, and can be considered as very efficient "artificial AP-nucleases".[5] These molecules were later shown to also interfere with the repair process of abasic sites by inhibiting AP-endonuclease activity. In addition, compound **1** significantly potentiates the antiproliferative action of the well-known anticancer alkylating agent BCNU (N,N'-bis(2-chloroethyl)-N-nitrosourea).[7] All these data suggest that molecules of the type "base-chain-intercalator" which specifically bind to abasic sites, might be of interest in anticancer chemotherapy.

To improve and modulate the biological properties of the heterodimers, it appears interesting to modify the geometry and possibly the strength of the drug-DNA interactions. High-field nmr and molecular modelling studies[8] of the interaction between **1** and a duplex oligonucleotide containing a stable analogue of the abasic site located opposite to a thymine residue in the complementary strand (Fig. 2) indicated that the purine ring of **1** inserts inside the abasic pocket, the acridine being intercalated at a two base pair distance and the amino linker lies inside the minor groove. The precise position of the purine moiety of the conjugate inside the abasic pocket could not be determined, however molecular modelling data favoured a Hoogsteen type hydrogen bonding with the thymine residue of the complementary strand. We designed molecule **6** in order to modify the geometry of the nucleic base of the conjugate in the complex and its hydrogen bonding ability. Introduction of the amino linker at

* Fax: +(33) (0)4 76 51 43 82; e-mail: Martine.Demeunynck@ujf-grenoble.fr

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C-8 of the purine would not disturb Watson-Crick and should even favour Hoogsteen base pairing by increasing charge density at N-7, it would also notably prevent steric clashes that may occur between the abasic site sugar and the linking chain attached to N-9 of the purine moiety. Meanwhile, it appears necessary to keep a substituent at N-9 to maintain the pKa of the purine. Substituted imidazo[1,2-e]purine fulfils these two conditions. We describe here the synthesis and preliminary DNA interaction study of a tricyclic imidazo[1,2-e]purine-acridine heterodimer.

Results and discussion. Preparation of imidazo[1,2-e]purine analogues has already been reported[9]. They were obtained by cyclization of 8-haloethyladenine. The reaction is not regioselective as cyclization takes place at both N-7 and N-9. We used a different strategy involving regioselective introduction of a polyamino linker at position 9[5] followed by intramolecular substitution of bromine at C-8. This requires bromination of the purine derivative **2**.

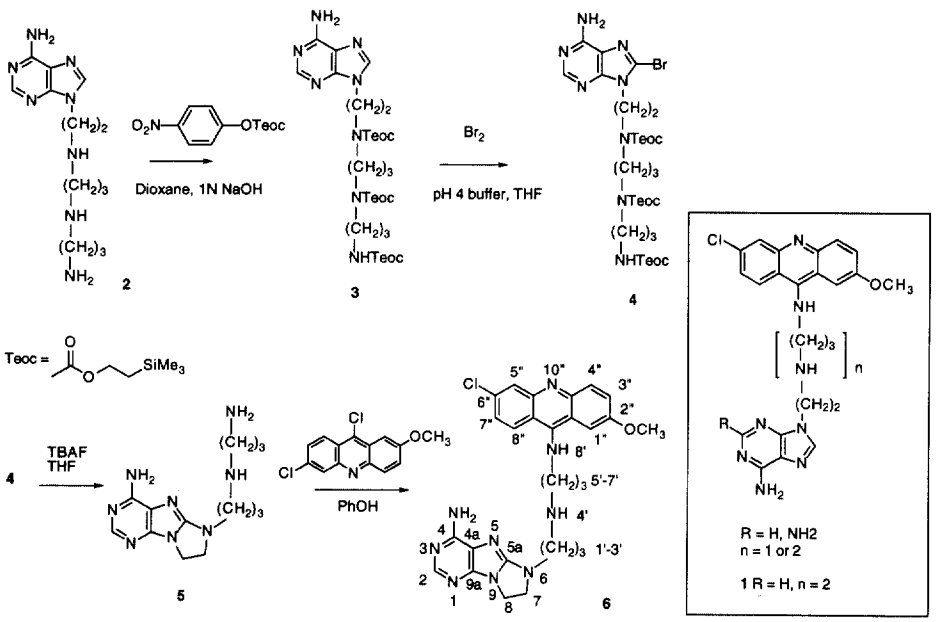


Figure 1

Direct bromination of **2** in a number of different conditions only occured in very low yields (10-20 %), it appeared necessary to protect the amino groups of the polyamino linker. Reaction with di-tert-butyl-dicarbonate (Boc_2O), benzyl chloroformate or benzoyl chloride gave complex mixtures of products. The 2-trimethylsilylethyl *p*-nitrophenyl carbonate (Teoc-ONp) was shown to be a reagent of choice to selectively protect the polyamino chain. Compound **3** was quantitatively prepared using a large excess of Teoc-ONp. The resulting oil[10] was then brominated in pH 4 buffer. Substitution at C-8 was confirmed by ^1H NMR, with the presence of only one aromatic signal (Ade-H2). The 8-bromopurine derivative **4** was obtained as an oil[11] and was used without further purification. Deprotection and cyclization were performed one-pot, by warming **4** in THF in the presence of tetrabutylammonium fluoride (TBAF). The reaction was monitored by hplc (diode array detector) with the formation of a polar compound (**5**) characterized by λ_{max} = 282 nm. Isolation of the polyamino intermediate **5**

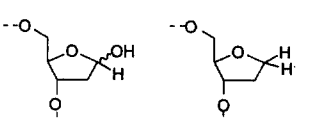
from the excess of TBAF salt was time and product consuming because of the high polarity of the reaction product. The crude reaction mixture was thus evaporated to dryness and the next step, introduction of the acridine nucleus, was done one-pot. Reaction with 6,9-dichloro-2-methoxyacridine in phenol afforded the heterodimer **6** as a yellow solid[12] in 22 % overall yield (starting from **3**). The tricyclic structure of the modified purine moiety was established by a detailed ^{1}H-^{13}C NMR analysis. In particular, HMBC experiments showed three characteristic heteronuclear ^{3}J couplings between C-5a and H-7, H-8 and H-1' that unambigously confirmed the imidazo[1,2-e]purine structure.

Before studying the interaction with DNA, it was important to determine the ionization state of the molecule at physiological pH. pK's of compound **6** were determinated both by potentiometry and UV/Visible titration. Using potentiometry, three values were found at 3.27, 7.02 and 8.92. By comparison with pK's of 8-aminoadenosine (pKa = 4.0)[13], pKa = 3.27 was attributed to the imidazopurine ring. UV/Visible titrations showed major modifications of the spectra between pH 7 and pH 10, indicating that pKa = 8.92 corresponds to the aminoacridine nucleus (pKa = 8.99 for 9-amino-3-chloro-7-methoxyacridine)[14] and therefore pKa = 7.02 to the secondary aliphatic amine of the linker. From these results, we can assume that at physiological pH, in the presence of DNA, the aminoacridine nucleus will be fully protonated, the aliphatic amine of the linker being partly protonated.

We then performed some preliminary experiments to probe the interaction with abasic sites. In a preceding paper[15], we showed that denaturation experiments (Tm measurement) constitute a method of choice to assay selective recognition of the abasic site by specific drugs. Thermal denaturations of a synthetic oligonucleotide duplex containing a stable analog of the abasic site (tetrahydrofuran, designated as X) and the corresponding conventional duplex were monitored by UV spectrophotometry.

Abasic site containing duplex (TX) GCGTGTGTGCG
CGCACXCACGC

Conventional duplex (TA) GCGTGTGTGCG
CGCACACACGC



Abasic site X

Figure 2

The presence of the abasic site in the duplex decreases the stability of the duplex (Tm = 36.7°C for duplex TX compared to Tm = 55.8°C for duplex TA). The interaction of an intercalating agent stabilizes the duplex and therefore increases the Tm value. Compound **6** was added to solutions of duplexes (TX and TA). Various ratios, from 0.5 to 2 equivalents of drug per oligonucleotide were used and the Tm were measured. Data were compared with those of reference compound **1** (Table 1).

Drugs	$r^a = 0.5$	$r^a = 1$	$r^a = 1.5$	$r^a = 2$
1[b]	5.2/15.7	8.8/21.6	11.1/24.1	12.1/-
6[c]	4.3/12.2	8.3/16.2	10.0/20.0	10.7/21.2

Table 1. ΔTm values obtained for the TA/TX duplexes. ΔTm = Tm (duplex in the presence of drug) - Tm (duplex alone). a) r = [drug]/[duplex]; b) Ref.15; c) Tm were measured at a total strand concentration of 7.69 μM and various ratio of the drug **6** at pH 7 in a buffer containing 10 mM sodium phosphate, 1 mM EDTA, 20 mM NaCl.

First of all, it is clear from these experiments that the presence of compound **6** stabilizes both duplexes (ΔTm > 0). The ΔTm values are higher for the abasic site containing duplex than for the conventional duplex. The values are lower for compound **6** compared to **1** for both TA and TX duplexes. This difference can be related to the state of protonation as at pH 7, compound **6** exists as a mixture of mono and bisprotonated species and compound **1** mainly as a mixture of bis and triprotonated forms.[15] Since we have previously demonstrated by high-field nmr [8] that **1** strongly and selectively binds to the abasic site, we can assume that the similarity of ΔTm variations for compound **6** is in favour of a selective binding of this new drug to abasic site.

The biological and pharmacological properties of compound **6** are currently under investigations.

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References and notes

1. Lefrançois, M.; Bertrand, J. M.; Malvy, C. *Mutation Res.* **1990**, *236*, 9-17.
2. Sancar, A. *Annu. Rev. Genet.* **1995**, *29*, 69-105.
3. O'Reilly, S.; Baker, S. D.; Sartorius, S.; Rowinsky, E. K.; Finizio, M.; Lubiniecki, G. M.; Grochow, L. B.; Gray, J. E.; Pieniaszek, H. J.; Donehower, R. C. *Ann. Oncol.* **1998**, *9*, 101-104.
4. Constant, J.-F.; Fkyerat, A.; Demeunynck, M.; Laval, J.; O'Connor, T. R.; Lhomme, J. *Anti-Cancer Drug Design* **1990**, *5*, 59-62.
5. Fkyerat, A.; Demeunynck, M.; Constant, J.-F.; Michon, P.; Lhomme, J. *J. Am. Chem. Soc.* **1993**, *115*, 9952-9959.
6. Fkyerat, A.; Demeunynck, M.; Constant, J.-F.; Lhomme, J. *Tetrahedron* **1993**, *49*, 11237-11252.
7. Barret, J. M.; Etiévant, C.; Fahy, J.; Lhomme, J.; Hill, B.T. *Anti-Cancer Drugs* **1998**, in press.
8. Coppel, Y.; Constant, J.-F.; Coulombeau, C.; Demeunynck, M.; Garcia, J.; Lhomme, J. *Biochemistry* **1997**, *36*, 4831-4843.
9. Hino, K.; Irie, A.; Uno, H. *Chem. Pharm. Bull., (Tokyo)* **1975**, *23*, 1696-1701.
10. Physical data for **3.** (oil) 95% yield. ^{1}H NMR (300 MHz, $CDCl_3$) 8.32 (s, 1H), 7.71 (s, 1H), 5.71 (s, 2H), 4.37 (m, 2H), 4.14-4.06 (m, 6H), 3.64 (m, 2H), 3.12-3.06 (m, 8H), 1.59 (m, 4H), 1.1-0.95 (m, 6H), 0.01 (m, 27H); MS (CI) *m/z* (%): 725 (100) [M^++1].
11. Physical data for **4.** (Oil) ^{1}H NMR (200 MHz, $CDCl_3$) 8.26 (s, 1H), 5.79 (s, 2H), 4.36 (m, 2H), 4.15-4.06 (m, 6H), 3.62 (m, 2H), 3.12-3.06 (m, 8H), 1.62 (m, 4H), 1.00-0.80 (m, 6H), 0.01 (m, 27H); MS (CI) 805 [M^++1, ^{81}Br], 803 [M^++1, ^{79}Br].
12. Physical data for **6.** mp 195 °C.^{1}H NMR (400 MHz, DMSO-d6) 8.36 (d, J = 9 Hz, H-8''), 7.88 (s, H-2), 7.84 (s, H-5''), 7.81 (d, J = 9 Hz, H-4''), 7.61 (s, H-1''), 7.40 (d, J = 9 Hz, H-3''), 7.28 (d, J = 9 Hz, H-7''), 6.41 (s, NH_2), 4.08 (H-8), 3.92 (s, OCH_3), 3.87 (t, H-7'), 3.84 (t, H-7), 3.25 (H-1'), 2.61 (H-5'), 2.50 (H-3'), 1.85 (H-6'), 1.70 (H-2'). ^{13}C NMR (100 MHz, DMSO-d6) 158.4 (C-5a), 154.9 (C-2''), 152.2 (C-4), 150.4 (C-9''), 148.9 (C-2), 148.3 (C-9a), 146.0 (C-4a''), 133.3 (C-6''), 130.0 (C-4''), 126.9 (C-5''), 126.6 (C-8'' and C-10a''), 123.8 (C-3''), 122.2 (C-7''), 121.8 (C-4a), 116.7 (C-9a''), 114.2 (C-8a''), 55.6 (OCH3), 52.0 (C-7), 48.0 (C-7'), 46.9 (C-5'), 46.5 (C-3'), 44.0 (C-1'), 39.5 (C-8), 30.1 (C-6'), 27.0 (C-2'); HRMS (positive FAB, NBA) *m/z* [M+H]$^+$ Calcd for $C_{27}H_{31}N_9O^{35}Cl$ 532.2340; Found 532.2341; UV/Vis (EtOH) λmax (lg ε) 221 (4.65), 269 (4.75), 342 (3.47), 359 (3.54), 418 (3.87).
13. Hovinen, H.; Glemarec, C.; Sandström, A.; Sund, C.; Chattopadhyaya, J. *Tetrahedron* **1991**, *47*, 4693-4708.
14. Albert, A. *The acridines*; second edition; Edward Arnold: London, **1966**, 161-172.
15. Berthet, N.; Constant, J.-F.; Demeunynck, M.; Michon, P.; Lhomme, J. *J. Med. Chem.* **1997**, *40*, 3346-3352.