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Synthesis of an Adenine-Pyridinaldoxime-Acridine Conjugate for Recognition of Abasic Site Lesions in DNA

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Abstract: Based on the DNA abasic site targeted artificial enzyme (ATAc **1**) previously developed in our laboratory, we designed and prepared a new molecule (**2**) incorporating a nucleophilic oxime function in the linker. A simple convergent strategy was used for the synthesis. The required pyridinic aldehyde derivative **9** was prepared by selenium oxide oxidation of the corresponding methylpyridine, and the two heteroaromatic moieties were successively condensed to afford the conjugate molecule. The oxime function was generated in the last step. © 1997 Elsevier Science Ltd.

INTRODUCTION

DNA abasic site is one of the non coding lesions occurring with high frequency by hydrolysis of the N-glycosidic bond[1a]. Unrepaired lesions may induce misincorporation of the opposite nucleotides during replication and promote mutagenesis[1b-d].

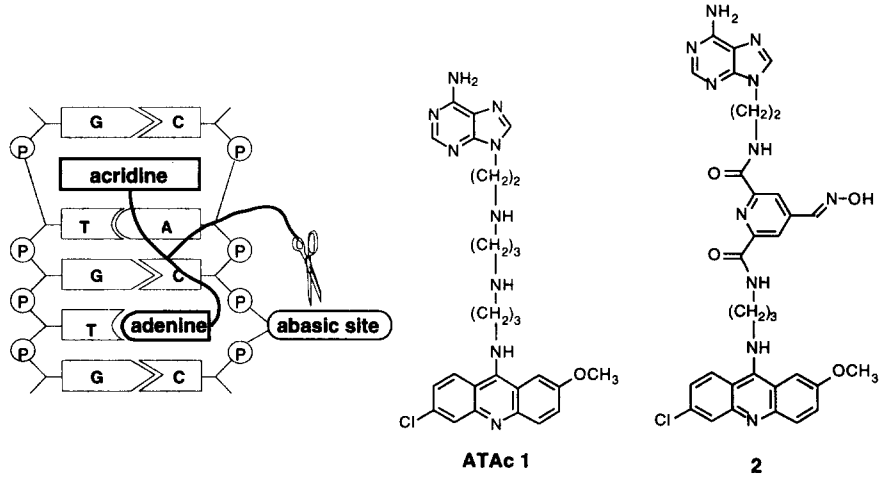

Figure 1 Schematic representation of abasic site cleavage (left) by ATAc **1** and the new target molecule **2**

In recent years, our group was interested in the development of new molecules which target the DNA abasic site and interfere with the repair of this important lesion[2-5]. Series of molecules have been designed,

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prepared and tested in this regard. Among them, one of the most promising molecules is the conjugate structure of three moieties, *i.e.* adenine, triamine and acridine (ATAc **1**). It was shown that ATAc is able to recognize the abasic sites and cleave the DNA strand at this position at a concentration as low as 10^{-9} mol/L by using plasmids containing abasic sites. During the SAR (Structure Activity Relationship) studies, a mechanism for this site specific cleavage was proposed. Accordingly, the adenine residue is positioned in the abasic cavity; this docking is stabilized by stacking interactions with the neighbouring bases and by hydrogen bonding with the unpaired base; the protonated acridine residue intercalates in a neighbouring site; and while one of the secondary amino group of the linking chain is protonated to interact with the negatively charged phosphates, the other acts as a base to cleave the abasic site by the well known β-elimination process (Figure 2).

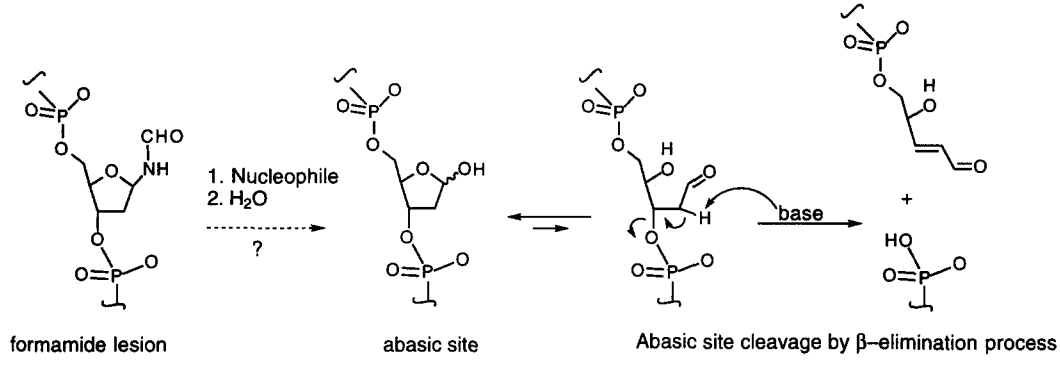


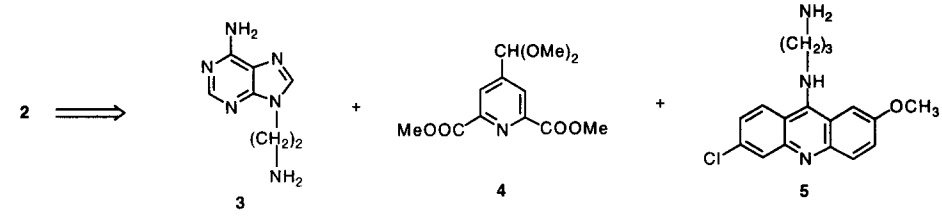
Figure 2 Formamide lesion and abasic site cleavage by β-elimination

More recently, a high field NMR study coupled with molecular modeling was carried out on an oligonucleotide (11mer) containing a stabilized abasic site analog[6]. The solution structure determination of the complex formed with the ATAc molecule revealed to be consistent with the above hypothesis and some additional structural details were obtained: *e.g.* the acridine moiety intercalates at a two base pair distance 5' to the abasic site.

Here we wish to report a new molecule that we designed to target the formamide lesions of DNA. The formamide lesion (Figure 2) is another kind of "alkali labile" abasic site which is observed predominantly upon γ-radiolysis of DNA[7]. High mutagenic nature of this lesion was demonstrated by Guy and Coll[8]. by study of *in vitro* replication using an oligonucleotide containing the formamide lesion as template. So it is interesting to design a molecule which recognizes and cleaves selectively this lesion. Preliminary tests in our laboratory[9] have shown that ATAc **1** does not cleave this lesion, while the hot piperidine treatment does. Although the cleavage mechanism of this "alkali labile" lesion seems never to have been examined, it is reasonable to assume that the first step is hydrolysis of the formamide function followed by hydrolysis of the aminosugar to the deoxyribose derivative and β-elimination of the 3'-phosphate. We made the hypothesis that a nucleophilic reagent could efficiently promote deformylation and trigger the cleavage[10]. We thus decided to synthesize molecule **2** keeping the two ends of ATAc **1** without modification, and replacing the triamino chain by the well studied pyridine aldoximate nucleophile[11]. In this article, we describe the synthesis of this new pyridine aldoxime (**2**).

RESULTS AND DISCUSSION

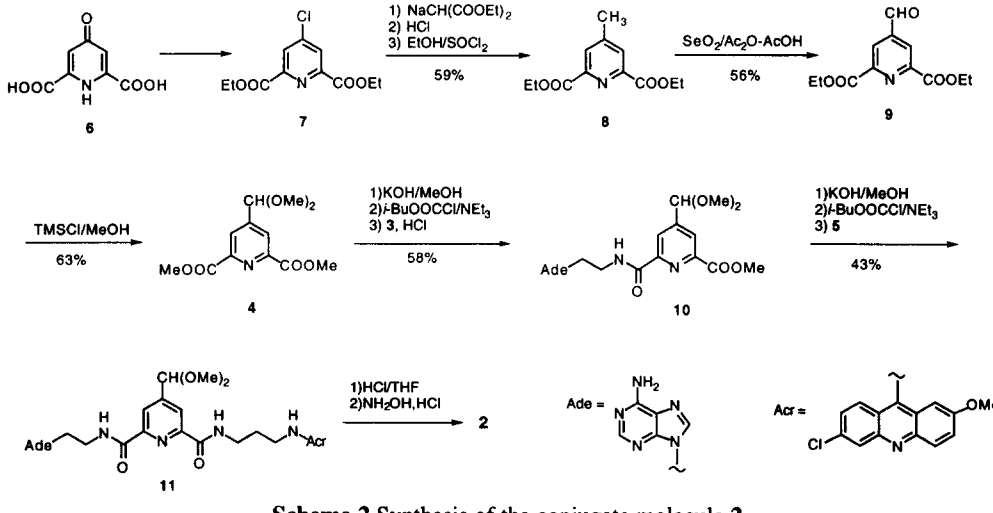
For the synthesis of **2**, we used a convergent strategy. The three parts of the molecule **3-5** were prepared separately and coupled successively. The oxime function was generated in the last step.



Scheme 1 Retrosynthesis of the conjugate molecule **2**

For the preparation of the required 4-formylpyridine dicarboxylate **9**, we developed a new route using selenium oxide oxidation of 4-methylpyridine dicarboxylate **8.** To our knowledge, this simple trifunctionnal pyridine **9** has never been described in the literature. We first prepared trimethyl pyridine 2,4,6-tricarboxylate from collidine[12] and examined its selective transformation to **9** without success. We next turned to an oxidative pathway using the 4-methylpyridine derivative **8** which is available in large scale using malonic ester synthesis. Thus chelidamic acid **6** was first transformed to the 4-chloropyridine diester **7**[13] then to 4-methylpyridine 2,6-dicarboxylic acid by malonate condensation followed by decarboxylation using the method described by Koenigs and Jaeschke[14] with slight modification. The Fischer esterification of the diacid gave the starting 4-methylpyridine diester **8**.

Upon treatment of the methylpyridine **8** with selenium oxide in mixed acetic acid and acetic anhydride, the corresponding aldehyde **9** was formed readily. It was separated from the minor amount of the corresponding acid by basic extraction. The aldehyde group was protected as the dimethylacetal using TMSCl treatment in methanol[15] during which the two ethyl ester groups were converted to the methyl esters to give **4**.



Scheme 2 Synthesis of the conjugate molecule **2**

The cristalline aminoethyladenine hydrochloride (**3**,HCl) was prepared according to the described method[16]. The aminopropylacridine **5** was prepared from commercially available dichloromethoxyacridine by treatment with an excess of diaminopropane.

The three components of the target molecule were successively coupled using *i*-butyl chloroformate as coupling reagent. Thus the symmetrical pyridine dicarboxylate **4** was hydrolyzed with a stoichiometric amount of KOH in methanol solution to the mono acid derivative which was coupled with the aminoethyladenine **3** to afford the amide **10**. Hydrolysis followed by another coupling of the resulting acid with the aminopropylacridine **5** in similar conditions yielded the desired conjugate acetal **11** which was purified by chromatography. Final deprotection of the aldehyde and oximation to **2** were carried out by usual methods.

No cleavage activity of this pyridinaldoxime **2** could be detected during the preliminary study on an oligonucleotide dodecamer containing a formamide lesion in the central position even at pH 11 (^{32}P labelling analysis by PAGE electrophoresis)[9]. This may indicate that the central pyridine dicarboxamide part has a too rigid structure to allow the oximate function to reach the appropriate reaction sites. Other interpretations can be formulated, among which reduced affinity of molecule **2** for DNA due to the loss of protonated secondary amino groups in the chain or reduced chemical reactivity of the oximate function which is not apt to promote the desired cleavage. Work is in progress to study the chemical reactivity of the formamide function using a simple deoxyribosylformamide.

In summary, we synthesized a new conjugate molecule incorporating a nucleophilic reagent, the oximate function. The convergent strategy used in this synthesis may be applied to prepare many other molecules with modifications of chain length, substitution, and functionnalities.

Experimental

General procedures:

All solvents or reagents were of reagent-grade quality and were used without further purification. Melting points were measured on a REICHERT THERMOVAR apparatus. Analytical thin layer chromatography (TLC) was performed on aluminium sheets pre-coated with silica gel (Merck Kieselgel 60 F_{254}, layer thickness 0.25 mm). Visualization was accomplished by UV light (254 nm) and/or phosphomolybdic acid solution. Preparative column chromatographies were carried out on silica gel (Merck Kieselgel, 230-400 mesh) according to the reference 17 unless otherwise indicated. ^{1}H NMR and ^{13}C NMR spectra were recorded on BRUKER WP80, AM200 and WP250 spectrometers. Spectra were referenced to the residual solvent peak. Infrared spectra were recorded on NICOLET "IMPACT 400" and PERKIN-ELMER 298 spectrometers. Mass spectra were recorded on DELSI-NERMAG R10-10. Elemental analysis were performed by "Service central de microanalyse du CNRS".

Diethyl 4-methylpyridine-2,6-dicarboxylate 8

To a suspension of NaH (10.0 g, 0.25 mol, 60% oil dispersion) in 350 mL toluene under argon, was added carefully 40.0 g (0.25 mol) of diethyl malonate with 50 mL of toluene. The exothermic reaction took place with hydrogen gas evolution and the temperature of the mixture was raised to 70°C. The resulting malonate salt was completely dissolved on heating at reflux and 22.2 g (86.2 mmol) of 4-chloropyridine **7** were added as solid using 50 mL of toluene for rinse. Reflux was maintained for 3 h. During this period, stirring

became difficult because of pasty oil separation at the bottom of the flask. The hot supernatant solution was removed by decantation and 420 mL of 4N HCl was added to the residual oil. The mixture was heated to reflux overnight. After cooling, the aqueous phase was washed with Et_2O and evaporated to dryness to afford about 35 g of yellow powder. This crude mixture was dissolved in 220 mL 1N NaOH (pH 8-9) then 50 mL of 2N HCl were added. After cooling, the precipitate was filtered and dried at 120°C for 3 h to give 14.6 g of the crude diacid. This material was directly used for Fischer esterification as follows.

13 mL of $SOCl_2$ were slowly added to 130 mL of EtOH. To this mixture, the above crude diacid (14.6 g) was added in one batch and the suspension was heated to reflux for 7 h. After cooling, insoluble material was filtered off and washed with EtOH, the combined filtrate and washings were evaporated and partitioned between 150 mL of CH_2Cl_2 and 50 mL of $NaHCO_3$ saturated solution. The organic phase was separated, washed with a saturated solution of $NaHCO_3$ and with brine, dried on Na_2SO_4 and evaporated to afford an oil (14.2 g, 59.8 mmol) which solidified on standing. Recrystallisation from pentane (150 mL) gave 8.92 g (37.6 mmol, 44 %). A second crop (3.10 g, 13.1 mmol, 15 %) of similar purity (by TLC) was obtained from the mother liquor. mp: 48-52°C; TLC/R_f: 0.25 (AcOEt/hexane, 1/1); IR (KBr): 3400, 3063, 2980, 2930, 2900, 1700, 1600, 1400, 1371, 1338, 1277, 1245, 1220, 1155, 1111, 1025, 991, 894, 866, 782, 739, 698 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$, δ ppm): 1.39 (t, 6H, J=7 Hz), 2.40 (s, 3H), 4.35 (q, 4H, J=7 Hz), 7.99 (s, 2H), ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 14.0, 20.8, 62.0, 128.4, 148.3, 149.8, 164.6.

Diethyl 4-formylpyridine-2,6-dicarboxylate 9

A mixture of diethyl 4-methylpyridine 2,6-dicarboxylate **8** (12.0 g, 50.6 mmol) and selenium oxide (7.50 g, 67.6 mmol) in acetic acid (40 mL) and acetic anhydride (10 mL) was heated at 120°C overnight. After cooling, the solution was filtered and evaporated. The crude product was dissolved in CH_2Cl_2 (250 mL), and washed with saturated $NaHCO_3$ solution (5×50 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo to afford the crude aldehyde (7.10 g, 28.3 mmol, 56%). mp: 112-116°C; TLC/R_f: 0.50 (AcOEt/hexane, 1/1); IR (KBr): 3395, 3070, 2995, 2970, 2820, 1710, 1600, 1570, 1470, 1410, 1375, 1340, 1275, 1215, 1155, 1110, 1020, 915, 865, 820, 780, 740, 700, 680 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$, δ ppm): 1.45 (t, 6H, J=7 Hz), 4.50 (q, 4H, J=7 Hz), 8.65 (s, 2H), 10.20 (s, 1H), ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 14.1, 62.6, 126.0, 143.7, 150.3, 163.6, 189.5. This product was used without further purification.

From the combined aqueous alkaline washings, careful acidification with 2N HCl followed by extraction using methanol/CH_2Cl_2 (1:9), drying on Na_2SO_4 and evaporation afforded 4.5 g (34 %) of crude acid. ^1H NMR (200 MHz, $CDCl_3$): δ ppm 1.45 (t, 6H, J=7 Hz), 4.50 (q, 4H, J=7 Hz), 5.00 (bs, 1H), 8.80 (s, 2H).

Dimethyl 4-dimethoxymethylpyridine-2,6-dicarboxylate 4

Trimethylsilylchloride (10.0 mL, 79 mmol) was added to a solution of aldehyde **9** (7.1 g, 28 mmol) in methanol (100 mL). The mixture was stirred overnight at room temperature. After removal of solvent *in vacuo*, the residue was dissolved in CH_2Cl_2 (200 mL), washed successively with a saturated solution of $NaHCO_3$ (50 mL) and with brine (50 mL) and dried over anhydrous Na_2SO_4, filtered and concentrated to yield 7.6 g of crude acetal. Flash column chromatography (silica gel, cyclohexane-AcOEt 60/40 v/v) gave 5.58 g (20.7 mmol, 73 %) of the analytically pure acetal **4**. mp: 87-92°C; TLC/R_f: 0.25 (AcOEt/hexane, 1/1); IR (KBr): 3400, 2950, 2830, 1710, 1600, 1560, 1440, 1330, 1270, 1250, 1220, 1140, 1100, 1060, 980, 890, 780, 735, 690, 620 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$, δ ppm): 3.34 (s, 6H), 4.01 (s, 6H), 5.48 (s, 1H), 8.35 (s, 2H), ^{13}C NMR

(50 MHz, $CDCl_3$, δ ppm): 52.7, 53.1, 100.3, 126.1, 148.5, 150.0, 164.9; Anal.: Calcd. for $C_{12}H_{15}NO_6$: C, 53.53; H, 5.61; N, 5.20. Found: C, 53.61; H, 5.61; N, 5.38.

Methyl 6-[2-(6-amino-9H-purin-9-yl)ethylcarbamoyl]-4-dimethoxymethylpyridine 2-carboxylate 10

KOH (101 mg dissolved in 2 mL of methanol) was added dropwise to a solution of the diester **4** (476 mg, 1.77 mmol) in THF (5 mL) at -10°C. The mixture was then stirred overnight at room temperature. The precipitated material was dissolved in water (50 mL). The aqueous solution was washed with Et_2O (10 mL), and the pH was then adjusted to 2 with 2N HCl. After extraction with CH_2Cl_2 (3×20 mL), the combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the monoacid (394 mg, 88% yield) which was used without further purification. mp: 100-104°C; IR: (KBr) 3630, 3460, 2940, 1730, 1240, 1060, 990 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$ δ ppm): 3.35 (s, 6H), 4.01 (s, 3H), 5.50 (s, 1H), 8.41 (s, 1H), 8.48 (s, 1H); MS (FAB+, nba) m/z: 256 $(M+H)^+$.

Triethylamine (0.2 mL, 1.3 mmol) was added to a solution of the above monoacid (324 mg, 1.27 mmol) in THF (10 mL). To the mixture maintained at 0°C, were successively added *i*-butyl chloroformate (173 mg, 1.3 mmol) and a suspension of the aminoethyladenine hydrochloride (**3**-HCl, 319 mg, 1.3 mmol, dried at 120°C overnight) and triethylamine (0.4 mL, 2.6 mmol) in DMF (10 mL). The mixture was stirred for 2 h at room temperature. After evaporation of the solvent, the solid was dissolved in CH_2Cl_2. The organic layer was washed with 1 N $NaHCO_3$ (20 mL) and then with water and brine. It was dried over anhydrous Na_2SO_4 and concentrated. The crude product was triturated in Et_2O and filtered off to afford compound **10**. (377 mg, 51% from **4**). mp: 192-198°C; TLC/R_f: 0.37 ($MeOH/CH_2Cl_2$, 1/9); IR (KBr): 3400, 3270, 3120, 2960, 1730, 1690, 1610, 1340, 1070 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$ δ ppm): 3.32 (s, 6H), 3.96 (m, 2H), 3.99 (s, 3H), 4.50 (t, 2H, J=6 Hz), 5.46 (s, 1H), 5.68 (bs, 2H), 7.79 (s, 1H), 8.28 and 8.39 (AB, 2H, J=1 Hz), 8.37 (s, 1H), 8.78 (bt, 1H, J=7 Hz); MS (FAB+, nba) m/z: 416 $(M+H)^+$; Anal.: Calcd. for $C_{18}H_{21}N_7O_5$,1/2H_2O: C, 50.94; H, 5.23; N, 23.10. Found: C, 50.95; H, 5.12; N, 22.80.

9-(3-amino-1-propylamino)-6-chloro-2-methoxyacridine 5

A mixture of 6,9-dichloro-2-methoxyacridine (2.9 g, 10.3 mmol), 1,3-diaminopropane (28.5 mL, 330 mmol) and phenol (8 g) was heated at 90°C for 4 h. After cooling, 400 mL of water were added and the resulting yellow precipitate was filtered and dried in a vacuum desiccator to yield 2.7 g (8.4 mmol, 82%). mp: 98-102°C; IR (KBr): 3150, 2920, 2840, 1720, 1630, 1510, 1440, 1330, 1240, 1170, 1070, 1025, 950, 920, 850, 820, 790 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$ δ ppm): 1.83 (quintuplet, 2H, J=6 Hz), 3.06 (t, 2H, J=6 Hz), 3.88 (s, 3H), 3.92 (t, 2H, J=6 Hz), 7.19 (dd, 1H, J=2 Hz and 9 Hz), 7.35 (d, 1H, J=2 Hz), 7.39 (dd, 1H, J=2 Hz and 9 Hz), 7.95 (d, 1H, J=9 Hz), 8.00 (d, 1H, J=2 Hz), 8.10 (d, 1H, J=9 Hz); MS (FAB+, nba) m/z: 316 $(M+H)^+$; Anal.: Calcd. for $C_{17}H_{18}N_3OCl$,3/4H_2O: C, 62.00; H, 5.97; N, 12.76. Found: C, 62.00; H, 5.80; N, 12.67.

N-[2-(6-amino-9H-purin-9-yl)ethyl]-N'-[3-(6-chloro-2-methoxyacridin-9-ylamino)propyl]-4-dimethoxymethylpyridine-2,6-dicarboxamide 11

To a suspension of **10** (247 mg, 0.57 mmol)) in MeOH/THF (5 mL, 1/1), was added 1.0 N methanolic KOH (0.64 mL) at room temperature. Complete dissolution took place within 1.5 h and the mixture was stirred

overnight. After careful neutralisation with 0.4 N methanolic HCl (1.4 mL) the solvents were evaporated *in vacuo* and residual methanol was eliminated by coevaporation with dry THF (3×10 mL). To the resulting solid mixture, were added dry THF (13 mL) and 0.5 N triethylamine in dry THF (1.1 mL, 0.6 mmol). On cooling on an ice bath, 0.5 N *i*-butyl chloroformate in dry THF (1.1 mL) was added and stirred for 5 min. The aminoacridine **5** (196 mg, 0.6 mmol) was added rapidly and the mixture was stirred for 45 min at 0°C then for 3 h at room temperature. After evaporation of the solvent *in vacuo*, the crude product was directly purified by column chromatography using silical gel (70-230 mesh) deactivated by addition of 15 wt% H_2O; eluant: 3.5 N methanolic NH_3/CH_2Cl_2 3/97 to 4/96. The recovered **11** was further purified by precipitation from $MeOH/CH_2Cl_2/Et_2O$ to afford 172 mg (43%) of yellow solid. mp: 135-140°C; TLC/R_f: 0.34 ($MeOH/CH_2Cl_2$, 1/9 + 1% NH_3 aq.conc.); IR (KBr): 3330, 2930, 2830, 1656, 1638, 1604, 1561, 1541, 1475, 1439, 1421, 1357, 1328, 1242, 1138, 1072, 927, 837, 801, 773, 678, 649, 570 cm^{-1}, ^1H NMR (250 MHz, $CD_3OD/CDCl_3$, 5/95, δ ppm): 1.9 (m, 2H), 3.3 (s, 6H), 3.7 (m, 4H), 3.81 (t, 2H, J=5 Hz), 4.01 (s,3H); 4.37 (t, 2H, J=5 Hz), 5.46 (s, 1H), 7.20 (dd, 1H, J=2 Hz and 9 Hz), 7.34 (dd, 1H, J=2 Hz and 9 Hz), 7.59 (d, 1H, J=2 Hz), 7.85 (d, 1H, J=9 Hz), 7.90 (s, 1H), 7.91 (d, 1H, J=2 Hz), 8.11 (d, 1H, J=9 Hz), 8.14 (s, 1H), 8.30 (d, 1H, J=1 Hz), 8.39 (d, 1H, J=1 Hz); MS (FAB+, nba) m/z: 668 $(M+H)^+$;

N-[2-(6-amino-9H-purin-9-yl)ethyl]-N'-[3-(6-chloro-2-methoxyacridin-9-ylamino)propyl]-4-hydroxyiminomethylpyridine-2,6-dicarboxamide 2

The acetal **11** (109 mg, 0.16 mmol) in 2 N HCl (23 mL) was stirred overnight at room temperature. Hydroxylamine hydrochloride (19 mg, 0.27 mmol) was then added. The pH was adjusted to 11 by addition of 4 N NaOH (10 mL). After addition of methanol (7 mL), stirring was continued for 1 h. The precipitated solid was filtered off and dried *in vacuo* to give a crude product (95 mg, 88%). A part of this material (55 mg)was purified by precipitation (H_2O/EtOH 75/25 v/v) to afford 31 mg of pure product **2** (56% yield of purification). mp : 167-173°C; IR (KBr) 3414, 3328, 2926, 2859, 1734, 1658, 1636, 1545, 1466, 1250, 1174, 1124, 1077, 1034, 936, 832, 798, 762, 685, 655, 574 cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6, δ ppm): 1.99 (m, 2H), 3.66-3.98 (m, 6H), 3.95 (s, 3H), 4.38 (m, 2H), 7.00-8.40 (m, 14H), 9.38 (m, 2H), 12.08 (bs, 1H); MS (FAB+, nba) m/z: 699 $(M+H)^+$;

References and Notes

1. (a) Lindahl, T.; Nyberg, B. *Biochemistry*, **1972**, *11*, 3610. (b) Loeb, L.; Preston, B. *Annual Review of Genetics*, **1986**, *20*, 201. (c) Gentil, A.; Margot, A.; Sarasin A. *Mutation Res.*, **1984**, *129*, 141. (d) Doetsch, P.W.; Cunningham, R.P.; *Mutation Res.*, **1990**, *236*, 173.
2. (a) Belmont, P.; Boudali, A.; Constant, J.-F.; Demeunynck, M.; Fkyerat, A.; Michon, P.; Serratrice, G.; Lhomme, J. *New J. Chem.*, **1997**, *21*, 47. (b) Berthet, N.; Boudali, A.; Constant, J.-F.; Decout, J.-L.; Demeunynck, M.; Fkyerat, A.; Garcia, J.; Laayoun, A.; Michon, P.; Lhomme, J. *J. Mol. Recognition*, **1994**, *7*, 99. (c) Fkyerat, A.; Demeunynck, M.; Constant, J.-F.; Lhomme, J. *Tetrahedron*, **1993**, *49*, 11237.
3. Constant, J.-F.; Fkyrerat, A.; Demeunynck, M.; Laval, J.; O'Connor, T.R.; Lhomme, J. *Anti-Cancer Drug Des.*, **1990**, *5*, 59.
4. Fkyerat, A.; Demeunynck, M.; Constant, J.-F.; Michon, P.; Lhomme, J. *J. Am. Chem. Soc.*, **1993**, *115*, 9952.

5. Constant, J.-F.; O'Connor, T.R.; Lhomme, J.; Laval, J. *Nucleic Acids Res.*, **1988**, *16*, 2691.
6. Coppel, Y.; Constant, J.-F.; Coulombeau, C.; Demeunynck, M.; Garcia, J.; Lhomme, J. *Biochemistry*, **1997**, *36*, 4831.
7. (a) Téoule, R. *Int. J. Radiat. Biol.*, **1987**, *59*, 589. (b) Téoule, R.; Bert, C.; Bonicel, A. *Radiat. Res.*, **1977**, 190. (c) Cadet, J.; Téoule, R. *Bull. Soc. Chim. Fr.*, **1975**, 879. (d) Cadet, J.; Téoule, R. *ibid.*, **1975**, 885. (e) Cadet, J.; Téoule, R. *ibid.*, **1975**, 891.
8. Guy, A.; Duplaa, A.-M.; Ulrich, J.; Téoule, R. *Nucleic Acids Res.*, **1991**, *19*, 5815.
9. Constant, J.-F.; Chavarot, M. Unpublished result. The oligonucleotide dodecamers containing a formamide lesion used for these studies were a generous gift of Dr Guy, A. (see ref. 7) to whom we are indebted.
10. Although base-catalyzed hydrolysis is most usual for non activated formamides, it has been shown that nucleophilic catalysis can also occur: Keillor, J.W.; Neverov, A.A.; Brown, R.S.; *J. Am. Chem. Soc.*, **1994**, *116*, 4669.
11. (a) Séguès, B.; Pérez, E.; Rico-Lattes, I.; Rivière, M.; Lattes, A. *Bull. Soc. Chim. Fr.*, **1996**, *133*, 925. (b) Terrier, F.; MacCormack, P.; Kizilian, E.; Hllé, J.-C.; Demerseman, P.; Guir, F.; Lion C. *J. Chem. Soc. Perkin Trans. 2*, **1991**, 153. (c) Epstein, J.; Cannon,Jr., P.L.; Michel, H.O.; Hackley,Jr., B.E.; Mosher, W.A. *J. Am. Chem. Soc.*, **1967**, *89*, 2937. (d) Edwards, J.O.; Pearson, R.G. *J. Am. Chem. Soc.*, **1962**, *84*, 16. (e) Jencks, W.P.; Carriuolo, J. *J. Am. Chem. Soc.*, **1960**, *82*, 1778. (f) Green, A.L.; Sainbury, G.L.; Saville, B.; Stansfield, M. *J. Chem. Soc.*, **1958**, 1583.
12. Syper, L.; Kloc, K.; Mlochowsk, J. *Tetrahedron*, **1980**, *36*, 123.
13. (a) Markees, D.G.; Kidder, G.W. *J. Am. Chem. Soc.*, **1956**, *78*, 4130. (b) Bradshaw, J.S.; Mass, G.E.; Lamb, J.D.; Izatt, R.M.; Christensen, J.J. *J. Am. Chem. Soc.*, **1980**, *102*, 467.
14. Koenigs, E.; Jaeschke, W. *Chem. Ber.*, **1921**, 1351.
15. Brook, M.A.; Chan, T.H. *Synthesis*, **1983**, 203.
16. Leonard, N.J.; Lambert, R.F. *J. Org. Chem.*, **1969**, *34*, 3241.
17. Still, W.C.; Kahn, M., Mitra, A. *J. Org. Chem.*, **1978**, *43*, 2923.

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