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Synthesis of an oligodeoxyribonucleotide adduct of mitomycin C by the postoligomerization method *via* a triamino mitosene

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Abstract

The cancer chemotherapeutic agent mitomycin C (MC) alkylates and cross-links DNA monofunctionally and bifunctionally *in vivo* and *in vitro*, forming six major MC-deoxyguanosine adducts of known structures. The synthesis of one of the monoadducts (**8**) by the postoligomerization method was accomplished both on the nucleoside and oligonucleotide levels, the latter resulting in the site-specific placement of **8** in a 12-mer oligodeoxyribonucleotide **26**. This is the first application of this method to the synthesis of a DNA adduct of a complex natural product. Preparation of the requisite selectively protected triaminomitosenes **14** and **24** commenced with removal of the 10-carbamoyl group from MC, followed by reductive conversion to 10-decarbamoyl-2,7-diaminomitosene **10**. This substance was transformed to **14** or **24** in several steps. Both were successfully coupled to the 2-fluoro-O^6- (2-trimethylsilylethyl)-deoxyinosine residue of the 12-mer oligonucleotide. The N^2-phenylacetyl protecting group of **14** after its coupling to the 12-mer oligonucleotide could not be removed by penicillinamidase as expected. Nevertheless, the Teoc protecting group of **24** after coupling to the 12-mer oligonucleotide was removed by treatment with $ZnBr_2$ to give the adducted oligonucleotide **26**. However, phenylacetyl group removal was successful on the nucleoside-level synthesis of adduct **8**. Proof of the structure of the synthetic nucleoside adduct included HPLC co-elution and identical spectral properties with a natural sample, and ^{1}H-NMR. Structure proof of the adducted oligonucleotide **26** was provided by enzymatic digestion to nucleosides and authentic adduct **8**, as well as MS and MS/MS analysis.

Introduction

Mitomycin C (**1**; MC[1]), an antitumor antibiotic, is used in clinical cancer chemotherapy.[2] Its cytotoxic and antitumor activity is attributed to its ability to alkylate DNA monofunctionally and bifunctionally, the latter mode resulting in DNA interstrand and intrastrand cross-links[3]. Six major DNA adducts have been isolated from *in vitro* systems, formed under biomimetic conditions, and their structures have been elucidated[4], including the DNA interstrand cross-

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Supporting Information Available: Figure S1: COSY NMR spectrum of synthetic adduct **8.** Figure S2: Ion spectrum of oligonucleotide **26** [M- 3H]$^{3-}$. This material is available free of charge via the Internet at http://pubs.

link (ICL), the first such adduct of a natural antibiotic[5] (Chart 1). The same six DNA adducts were shown to form in tumor cells treated with MC[6]. The ICL was also isolated from rat liver DNA of animals injected with the drug[5]. The structures of the six MC-DNA adducts illustrate that the exclusive target of alkylation of DNA by MC is the guanine base. Individual structure-activity relationships of multiple DNA adducts generated by a single agent have been investigated mostly in the case of organic mutagens and carcinogens[7]. In general, such studies utilize synthetic oligonucleotides bearing a specific adduct at a unique position of their base sequences. The MC adducts present an opportunity for similar studies, enabling in this case direct comparisons of biological effects of the various DNA adducts of a cancer chemotherapeutic agent. Using this approach, comparison of the effects of monoadducts and interstrand cross-links of MC, its interstrand and intrastrand cross-links, DNA adducts of MC and DNA adducts of the MC metabolite 2,7-diaminomitosene[8] (**2**; 2,7-DAM) formed *in situ* in MC-treated cells[4], for example, would be possible. To date, we carried out a comparison between the mutagenic and cytotoxic effects of MC- N^2-guanine monoadduct **3**[9a] and compared these properties with those of the 2,7-DAM-N^7-guanine adduct **7**.[9b]

Synthesis of most of the six MC adducts (Chart l) incorporated in oligonucleotides has been accomplished by the biomimetic route, consisting of the alkylation reaction of MC with a short DNA duplex of appropriate sequence in the presence of a reductive MC-activating agent. Varying the activation conditions leads to different adducts[10]. This approach has been successfully applied in the case of adducts **3**[11], **4**[11], **5**[12], and **7**[13]. However, the method is usually inefficient, due to the difficulty of purification of the product to homogeneity. Furthermore, adducts **6** and **8** are formed in very low yield by the biomimetic approach to be practical.

We report here the first alternative access to one of the adducts of MC, based on organic synthetic methods, featuring the postoligomerization/convertible nucleoside approach[14a,b,c] in which the normal nucleophile-electrophile relationship of the DNA nucleoside and the drug is reversed by using an amine derivative of the latter and 2-fluorodeoxyinosine as the DNA target site. Specifically, we describe a synthesis of monoadduct **8** on both the nucleoside and oligonucleotide levels. In view of the highly sensitive functional groups of the MC molecule its selective conversion to the requisite amine derivatives **14** and **24** took six steps to be accomplished. The adduct described herein is, to the best of our knowledge, the most complex DNA adduct synthesized by the postoligomerization strategy to date. This strategy was necessitated by the fact that although adduct **8** is a major adduct in MC-treated tumor cells it is formed only in trace quantities *in vitro* by biomimetic reactions. Assignment of its structure was previously based only on absorption spectrophotometric and mass spectroscopic data, mostly on material obtained from cells[4]. We present now definitive ^{1}H-NMR characterization of adduct **8**.

Adduct **8**, along with adduct **7**, is the product of reductive alkylation of DNA by 2,7-DAM (**2**), the reduced metabolite of MC. 2,7-DAM is the major product of the reductive activation of MC *in vivo*[15a], as well as the enzymatic or chemical reduction of MC *in vitro* (Scheme 1) [15b]. Its 10-carbamoyloxy function is activated for displacement by a DNA nucleophile after a new reduction of the quinone function *in situ*[13]. DNA from 2,7-DAM-treated tumor cells contains adducts **7** and **8**, formed at relatively high frequency[4]. Yet, surprisingly, 2,7-DAM is essentially non-cytotoxic to the same cells, in contrast to the parent MC[16]. Consistent with this finding, in a structure –activity study, adduct **7** was found to be non-cytotoxic and non-mutagenic in transfection experiments and could be by-passed by high-fidelity DNA polymerases *in vitro*[9b] . The present synthetic approach to the previously unavailable sister adduct **8** will provide a substrate to examine the biological and structural properties of **8** in parallel with its major groove adduct counterpart **7**. It will also be interesting to compare

properties of **8** with those of the other minor groove guanine-N^2 monoadducts of MC, **3** and **4**

Results

Synthesis of the 2-amino-protected 2,7,10-triamino-10-decarbamoylmitosene 14 from MC (Scheme 2)

MC was converted in two steps to 2,7-diamino-10-decarbamoyl mitosene **10**[15b,c] followed by four additional steps to obtain **14** protected at its 2-amino group by a phenylacetyl group. Conversion of the 10-hydroxyl function to the 10-amine (**12**→ **13**→ **14**) proceeded smoothly by the Mitsunobu reaction[17]. The phenylacetyl protecting group has been shown previously to be removable from oligonucleotide bases enzymatically by penicillin amidase[18]. Deprotection of **14** to the free amine **15** was accomplished accordingly, but since the necessary intermediate in the desired transformations was **14**, the free mitosene **15** was not rigorously characterized. Alternatively, access to compound **14** was also possible *via* the conversion of **12** to the 10-azide **21** followed by a Staudinger reaction to give **14**. Both synthetic routes were explored and were found to give similar overall yields (40%).

Synthesis of the adducted nucleoside 8 (Scheme 3)

was accomplished by reaction of **14** with 2-fluoro-O^6-(2-p-nitrophenylethyl)-deoxyinosine **16**[19] to give **17,** followed by two consecutive deprotection steps: first, removal of the 2-p-nitrophenylethyl group by DBU from the nucleobase[19], then hydrolysis of the phenylacetamide **18** by penicillin amidase to give the free amine **8.** This substance was shown to be identical with adduct **8** isolated from MC-treated tumor cells[6] and from a biomimetic reaction *in vitro*[4], with respect to their co-elution by HPLC, and its mass, UV and CD spectra (Figure 1). Its ^{1}H-NMR and COSY spectra (Figure 2 and S1 respectively) support the previously derived structure as **8**[4].

Coupling of 14 to the 12-mer oligonucleotide 5'-CTAGTGXTATCC (19a; X =2-fluoro-O^6-(2-trimethylsilylethyl)-deoxyinosine[20]; Scheme 4)

was accomplished using DMSO as solvent and diisopropyl ethylamine as a base, with incubation at 42°C, for 72 hours. The crude mixture was hydrolyzed by mild acid to remove the TMSE[1] protecting group (**20a**)[14c,d]. The mixture was then neutralized to pH 7 and the N^2-phenylacetyl-protected mitosene-oligonucleotide adduct **20a** was purified by HPLC. Attempts to remove the phenylacetyl protecting group from the mitosene residue in oligonucleotide **20a** by penicillin amidase have failed repeatedly. In order to verify that this failure was independent of the sequence we used, we also coupled **14** to a different 12-mer oligonucleotide 5'-GCTAGCXAGTCC-3' (**19b**)[20]. Deprotection of the analogous alkylated oligo (**20b**) also failed consistently. These failures necessitated the synthesis of a triaminomitosene with a more suitable 2-amino protecting group that would be removable from the oligonucleotide adduct. This was accomplished as follows:

Synthesis of a new N^2-protected triaminomitosene 24 (Scheme 5)

Mitosene **21** was hydrolyzed by penicillin amidase to **22**. The free 2-amino group of **22** was then outfitted with the new protecting group trimethylsilylethoxycarbonyl (Teoc)[21] to give **23**. Reduction of the 10-azido group of **23** to the 10-amine gave the desired protected triaminomitosene derivative **24,** intended for the coupling to oligonucleotides.

Coupling of the new triaminomitosene 24 and the 12-mer oligonucleotide 5'-CTAGTGXTATCC (19a; X =2-fluoro-*O*^{6}-(2-trimethylsilyl-ethyl)-deoxyinosine)[20] and successful deprotection to the adducted oligonucleotide 26 (Scheme 6)

Compounds **24** and **19a** were incubated in DMSO in the presence of diethylamine for 72 hours at RT. HPLC indicated that the coupling yielded 80 % of a product, presumably **25** as it showed absorbance at 315 nm. Oligonucleotide-type materials were isolated through ethanol precipitation. Deprotections at the guanine-O^6 and mitosene-N^2 positions were performed in one step using $ZnBr_2$. The final product **26** was isolated by Sephadex G-25 gel filtration followed by HPLC purification in 57 % overall yield.

Verification of the structure and purity of the adducted oligonucleotide 26

The oligonucleotide product **26** was isolated by HPLC and a mass spectrum was obtained confirming the identity of the correct site-specifically modified oligonucleotide. Negative ESI-MS results showed that the deconvoluted mass of **26** was 243 Da higher than the calculated mass of the unmodified 5'-CTAGTGGTATCC which is consistent with the presence of adduct **8** in the substrate. Moreover high-resolution negative ESI-MS results revealed that the deconvoluted mass of the molecular ion 3877.74444 differed from the calculated *m/z* of 3877.74104 for the corresponding peak by only 0.8 ppm (Figure S2). We next characterized the above oligonucleotide by MS/MS (Figure 3) as was described previously in an analogous case[14b]. The product-ion spectrum of the $[M-3H]^{3-}$ ion (*m/z* 1292) of the oligonucleotide **26** showed the formation of w_n ions, that is, w_1^-, w_3^-, w_5^{2-}, w_6^{2-}, w_8^{2-} and [a_n-Base] ions, that is, $[a_2\text{-T}]^-$ $[a_3\text{-A}]^-$ $[a_4\text{-G}]^-$ $[a_6\text{-G}]^-$ $[a_7\text{-}\mathbf{8}]^-$ $[a_{11}\text{-T}]^-$ ions (nomenclature for fragments ions follows that reported by McLuckey et al.[22]). The measured masses for the w_1, w_3 and w_5 ions were the same as the calculated masses for the corresponding ions of the unmodified oligonucleotide, whereas the w_6 and w_8 ions exhibited 243 Da higher in mass than the calculated corresponding fragments formed from the unmodified d(CTAGTGGTATCC). These results are consistent with the presence of adduct **8** at the seventh position in the oligonucleotide. The above conclusion is further substantiated by the observed masses for the [a_n-base] ions. In this respect, the measured masses for the $[a_{11}\text{-T}]^{6-}$ ion is 243 Da higher whereas the measured masses of the $[a_2\text{-T}]^-$ $[a_3\text{-A}]^-$ $[a_4\text{-G}]^-$ $[a_6\text{-G}]^-$ $[a_7\text{-}\mathbf{8}]^{2-}$ are the same as the calculated ones of the corresponding fragment ions for the unmodified d (CTAGTGGTATCC). This technique confirmed the site of adduct **8** incorporation.

In addition, oligonucleotide **26** was submitted to enzymatic digestion to nucleosides followed by HPLC analysis. The chromatogram of the digest established the presence of the mitosene-adducted nucleoside **8**. This was further validated by the coelution of an authentic sample of **8** with this digestion product (Figure 4). Furthermore, we integrated the peak areas for adduct **8**, dC dG and dT in the HPLC traces of the digest of oligonucleotide **26** (Figure 4a) and estimated the molar ratios with the consideration of the molar extinction coefficients (E_{260}) of the three nucleosides. In the case of adduct **8**, a correction for E_{260} was done by adding 5189 L mol^{-1} cm^{-1} (E_{260} of 2,7-DAM) to that of dG [9b]. It turned out that the calculated molar ratios of **8**:dC:dG:dT were 1:2.8:2:4.3 which are consistent with the presence of one adduct **8**, three dC, two dG and four dT residues in oligonucleotide **26**. Because of the partial deamination of dA to dI during enzymatic digestion, the peak area for dA was not determined.

Discussion

The synthesis of a suitable aminomitosene for the postoligomerization coupling with nucleoside **16** and oligonucleotide **19** from 2,7-diamino-10-decarbamoyl-mitosene **10** required two main synthetic imperatives: (i) conversion of the 10-hydroxyl group to a 10-amino group and (ii) suitable protection for the 2-amino group, so that the coupling would occur solely at the 10-position. The protecting group also had to be removable under mild conditions so as

not to damage the mitosene moiety and be compatible with the presence of amines, i.e., base resistant.

Both tasks demanded that we explore the chemical reactivity of 2,7-diamino-10-decarbamoyl-mitosene **10**. This was a difficult undertaking as MC derivatives have several highly reactive functional groups; therefore it is hard to find suitably selective protection and deprotection methods applicable to these molecules in general.

We first chose the phenylacetyl group as the protecting group for the 2-amino position and synthesized the amino mitosene **14** from MC. The phenylacetyl group has been previously used by other research groups to protect peptides and oligonucleotides[23]. This group was utilized for the protection and deprotection of amino functions of nucleobases, since being removable by enzymatic hydrolysis around neutral pH it offered an alternative route to the acidic or basic conditions required for the removal of the established blocking groups. 2,7-Diamino-10-decarbamoyl-mitosene **10** was submitted to phenylacetylation using phenylacetic anhydride. The anhydride was found to be highly reactive and led to extremely short reaction times. Both the hydroxyl and amino group were acylated in less than 5 min; after longer times a new yellow compound was formed, probably resulting from hyperacylation of the mitosene moiety. Selective removal of the phenylacetyl group from the 10-hydroxyl group was executed using ammonia in methanol.

Two routes for the conversion of the 10-hydroxyl function of **12** to the 10-amine of **14** were explored and were found to give similar yields. One proceeded smoothly by the Mitsunobu reaction. Formation of the N^7- iminophosphorane was observed in some cases due to the presence of excess triphenylphosphine. However, acidic hydrolysis efficiently converted the iminophosphorane back to the free 7-amino group. The second route we investigated was the conversion of the 10-hydroxyl group to an azido group. The hydroxyl group was first mesylated. The nucleophilic substitution of the mesylate by the azido group proceeded smoothly in DMSO to yield **21**. Reduction of the azido group to the amino group was achieved *via* a Staudinger reaction in ammonium hydroxide to give **14**. The Staudinger reaction did not proceed without the presence of ammonia in the reaction mixture.

The reactivity of the aminomitosene **14** was sufficient for the synthesis of adduct **17** which was then deprotected enzymatically to adduct **8** using penicillin amidase. Previous elucidation of the structure of adduct **8** isolated from sources of biomimetic material was based primarily on the difference UV spectral method[24], MS and chemical tests[4]. However, due to the scarcity of material, no NMR data of the adduct has been obtained and definite proof of its structure was still lacking. The present organic synthesis of adduct **8** enabled the unambiguous characterization of this adduct.

The condensation reactions of **14** with oligonucleotides **19a** and **19b** occurred in more dilute solutions than the reaction with nucleoside **16** ([amine]=0.98 M for the nucleoside versus [amine]=0.3 M for the oligonucleotides). As a result, the rates were significantly lower and maximal couplings were achieved after 72 h. Because of the ease with which the enzymatic deprotection occurred at the nucleoside level, as well as of the reported enzymatic deprotection of phenylacetylated bases in oligonucleotides,[18,23] a similar outcome was anticipated for the oligonucleotide coupling product, perhaps at a slower rate. However, when oligonucleotide **20a** was submitted to penicillin amidase treatment, the deprotection failed repeatedly. We varied the reaction conditions (i. e. temperature, enzyme concentration, oligonucleotide concentration) and the sequence of the substrate (**20a** *vs.* **20b**) to no avail. Although some removal of the phenylacetyl group was detected, this was accompanied by degradation, as HPLC of the reaction mixture revealed the appearance of several new uncharacterized alkylated oligonucleotides. Evidently, the efficacy of the enzymatic transformation was subtly and

strongly dependent on the type of substrate. We concluded that the enzymatic deprotection was not appropriate at the oligonucleotide level and this approach was abandoned.

This repeated failure prompted us to look for new protecting groups. A proper protecting group had to be removable under non-basic (since the coupling occurs in the presence of base) and non- acidic conditions (to prevent depurination). Our focus shifted to the Teoc group which can be removed with $ZnBr_2$ under mild conditions. The Teoc group has been recently used to protect nucleosides in oligonucleotide synthesis.[21] The synthesis of mitosene **24** was performed according to Scheme 5 as attempts to selectively protect the 2-amino group of compound **10** with the Teoc group in one step was unsuccessful. The protected mitosene **24** was coupled to oligonucleotide **19a**. The coupling occurred readily as monitored by HPLC. The product appeared as a new peak with an absorbance at 320 nm, serving evidence of the incorporation of the mitosene moiety into the oligonucleotide. The crude modified oligonucleotide **25** was subsequently subjected to deprotection. $ZnBr_2$ was found to be the best reagent leading to complete and rapid deprotection of the Teoc group at the nucleoside level.[21] These conditions also removed the O^6 -TMSE group offering the advantage of complete deprotection of oligonucleotide **25** at the mitosene-N^2 and guanine-O^6 positions in a single step. This limited the number of transformations performed on oligonucleotide **25** hence increasing the overall yield.

Conclusion and Significance

The aim of the present work was to prepare a mitomycin C (**1)** derivative suitable for incorporation of the known MC-deoxyguanosine adduct **8** in oligonucleotides, using the postoligomerization adduct synthesis method[14]. This was accomplished by conversion of MC (**1**) to the N^2-protected triamino-10-decarbamoyl-mitosene **24** in six steps, followed by coupling of this mitosene to the O^6-protected 2-fluorodeoxyinosine residue of oligonucleotide **19a.** Chemical removal of both protecting groups of the product **25** in one step yielded the final product **26.** This is the first report, to our knowledge, of an application of postoligomerization synthesis to a DNA adduct of an antitumor antibiotic natural product. The mitosene derivative **14** was utilized for an authentic synthesis of deoxyguanosine adduct **8**, which was previously not characterized rigorously[4].

This work extends the preparative availability of known MC adducts on the oligonucleotide level, enabling further studies of adduct structure and adduct structure-activity relationships. Our results raise the possibility that the last oligonucleotide-level MC adduct, intrastrand cross-link **6** (Chart 1) that is still unavailable for such studies, may be synthesized by a similar postoligomerization approach.

Experimental Section

Materials

Mitomycin C was obtained from Bristol-Myers Squibb, Wallingford, CT and from Kyowa Hakko Kogyo Co., Ltd., Japan. Synthetic oligonucleotides containing O^6-protected 2-fluorodeoxyinosine were a kind gift of Dr. Carmelo Rizzo from Vanderbilt University, and further purified by us as described below. Reagents were obtained from commercial sources and were used without further purification. Thin layer chromatographic analyses were carried out on 250 μm silica gel plates containing a fluorescent indicator and, if necessary, spots were visualized with I_2. Column chromatographic purifications were performed using 200−300 mesh silica gel. All reactions were carried out under an atmosphere of Ar unless otherwise noted. ^{1}H-NMR spectra were obtained at 500 MHz and are referenced to residual protonated solvent. Chemical shifts are reported in parts per million and coupling constants are in hertz (Hz). Carbon-13 data was recorded at 125 MHz. Mass spectra of modified oligonucleotides

and HRMS of all compounds were recorded using Agilent Technologies 6210 TOF (HPLC: Agilent 1200), except for oligonucleotide **26** the mass spectrum of which was recorded using Agilent Technologies 6520 Q-TOF (HPLC: Agilent 1200), at Hunter College Mass Spectrometry Facility. Synthetic oligonucleotides were purified by C-18 reverse phase HPLC in NH_4HCOO (0.1 M in water)/acetonitrile with a diode array detector monitoring at 260 and 320 nm. The following gradient was used: initial condition 99% NH_4COO (0.1 M in water)-1% acetonitrile, then 60 min linear gradient to 80% acetonitrile, 5 min at 80% acetonitrile followed by 5 min linear gradient to initial conditions. The conventional numbering system is used for the MC and mitosene[8] moities and the purine carbons are numbered 1−9 also as per convention.

2,7-Diamino-10-decarbamoylmitosene[8] (10)

Palladium over charcoal (71.3 mg) was added to a solution of 10-decarbamoyl mitomycin C[15c] (170.7 mg, 0.587 mmol) in deaerated methanol (18 mL). H_2 gas was bubbled through the solution while acetic acid (54.8 μL, 57.5 mg, 0.957 mmol) was added by a syringe. H_2 gas was bubbled gently for a additional 10 min. TLC indicated the disappearance of all starting material. The reaction mixture was filtered through Celite and concentrated *in vacuo*. The product (136 mg, 90% yield) was isolated by silica gel chromatography (SiO_2: 10% to 15 % MeOH in CH_2Cl_2).^{1}H NMR (methanol-d_4) δ 1.82 (s, CH_3, 3H), 2.73 (dd, H_1, J=4.5, 16.5 Hz, 1H), 3.23 (dd, H_1, J=3.8, 16.5 Hz, 1H), 3.99 (dd, H_3, J=4.3, 12.9 Hz, 1H), 4.24 (m, H_2, 1H), 4.41 (dd, H_3, J=6.7, 12.9 Hz, 1H), 4.60 (s, CH_2, 2H). ^{13}C NMR (methanol-d_4) δ 6.8 (1C, CH_3), 31.7 (1C, CH_2), 53.9 (1C), 57.9 (1C), 58.7 (1C), 104.6 (1C, C=C), 112.1 (1C, C=C), 121.8 (1C, C=C), 128.6 (1C, C=C), 139.7 (1C, C=C), 147.5 (1C, C=C), 177.8 (1C, CO), 178.5 (1C, CO). HRMS *m/z* calcd for $C_{13}H_{15}N_3O_3Na$ $[M+Na]^+$ 284.1005, found 284.1026.

2,7-Diamino-N^2-phenylacetyl-10-decarbamoylmitosene (12)

2,7-diamino-10-decarbamoylmitosene (10 mg, 0.039 mmol) was dissolved in 1 mL of dry pyridine and the reaction vessel was sonicated. Phenylacetic anhydride (43.8 mg, 0.42 mmol) was added. TLC indicated the disappearance of all starting material after 10 min. The reaction mixture was diluted with ethyl acetate and washed with water, dilute HCl, and $NaHCO_3$. Drying ($MgSO_4$) and evaporation of solvents gave a red residue which was dried in a desiccator under vacuum. The residue was redissolved in 2.5 mL of 7 N ammonia in methanol. The mixture was stirred overnight at room temperature. The resulting product was diluted with ethyl acetate, washed with water, satd. $NaHCO_3$ and once more with water. The organic phase was dried ($MgSO_4$). Evaporation of solvents gave a red residue. The final product was purified by chromatography (SiO_2: 10% MeOH in CH_2Cl_2). (13 mg, 89 % yield). ^{1}H NMR (methanol-d_4) δ 1.81 (s, CH_3, 3H), 2.81 (dd, H_1, J=4.5, 16.5 Hz, 1H), 3.25 (dd, H_1, J=7.5, 16.5 Hz, 1H), 3.52 (s, CH_2, 2H), 4.07 (dd, H_3, J=3.5, 12.5 Hz, 1H), 4.44 (dd, H_3, J=7.5, 12.5 Hz, 1H), 4.69 (s, CH_2, 2H), 4.96 (m, H_2, 1H), 7.30 (m, H_{ar}, 5 H). ^{13}C NMR (methanol-d_4) δ 6.8 (1C, CH_3), 29.4 (1C, CH_2), 42.2 (1C), 52.3 (1C), 52.6 (1C), 55.5 (1C), 104.7 (1C, C=C), 116.8 (1C, C=C), 121.8 (1C, C=C), 126.5 (1C, Ph), 128.2 (2C, Ph), 128.7 (2C, Ph), 135.4 (1C, Ph), 138.3 (1C, C=C), 147.5 (1C, C=C), 172.6 (1C, CO), 177.8 (1C, CO), 178.9 (1C, CO). HRMS *m/z* calcd for $C_{21}H_{22}N_{22}N_3O_4$ $[M+H]^+$ 380.1610, found 380.1614.

Synthesis of 13

A mixture of **12** (20 mg, 0.0527 mmol), triphenyl phosphine (40.16 mg, 0.158 mmol) and phtalimide (62.1 mg, 0.422 mmol) was dissolved in dry DMF. *t*-Butyl-azodicarboxylate was added (36.46 mg, 0.158 mmol). The solution was stirred at room temperature for 2 h. TLC analysis showed disappearance of the starting material. The reaction mixture was diluted with ethyl acetate and washed three times with water. Drying (Na_2SO_4) and evaporation of solvents gave a crude purple oil. The final product was isolated by chromatography (SiO_2: 20% CH_2Cl_2 80% ethyl acetate) to yield 15 mg of product (56% yield).^{1}H NMR ($CDCl_3$) δ 1.79

(s, CH_3, 3H), 2.59 (dd, H_1, *J*=4.1, 16.5 Hz, 1H), 3.11 (dd, H_1, *J*=7.6, 16.5 Hz, 1H), 3.57 (s, CH_2, 2H), 3.99 (dd, H_3, *J*=4, 13.3 Hz, 1H), 4.40 (dd, H_3, *J*=6.9, 13.4 Hz, 1H), 4.88 (d, *J*=9.4 Hz, 1H), 4.88 and 5.01 (AB quartet, CH_2, *J* =15.5 Hz, 2H), 5.07 (m, H_2, 1H), 7.24−7.35 (m, H_{ar}, 5 H), 7.75 (dd, *J* =3.15, 5.5 Hz, 2H) and 7.87 (dd, *J* =3.1, 5.5 Hz, 2H). HRMS *m/z* calcd for $C_{29}H_{25}N_4O_5$ $[M+H]^+$ 509.1825, found 509.1810.

10-Azido-2,7-diamino-*N*^{2}-phenylacetyl-10-decarbamoylmitosene (21)

Compound **12** (46 mg, 0.121 mmol) was diluted in 1 mL of dry pyridine and cooled to 0°. Methanesulfonyl chloride (60 μL, 88.44 mg, 0.772 mmol) was added dropwise and the reaction mixture was warmed to room temperature and stirred for 2 h. TLC indicated the disappearance of all starting material. Solvents were evaporated and the residue was dried in a desiccator. The crude material was dissolved in dry DMSO and dry sodium azide (100 mg, 1.53 mmol) was added. The reaction mixture was heated at 100° for 2.5 h. The resulting red residue was purified by flash chromatography (SiO_2: 2% to 10% MeOH in CH_2Cl_2). (23.5 mg, 48 % yield). ^{1}H NMR (methanol-*d*$_4$) δ 1.79 (s, CH_3, 3H), 2.79 (dd, H_1, *J*=4.3, 16.5 Hz, 1H), 3.24 (dd, H_1, *J*=7.5, 16.5 Hz, 1H), 3.49 (s, CH_2, 2H), 4.08 (dd, H_3, *J*=4.2, 13.1 Hz, 1H), 4.44 (dd, H_3, *J*=7.1, 13.1 Hz, 1H), 4.45 (s, CH_2, 2H), 4.96 (m, H_2, 1H), 7.25 (m, H_{ar}, 5 H). ^{13}C NMR (methanol-*d*$_4$) δ 6.8 (1C, CH_3), 29.5 (1C), 39.0 (1C), 42.2 (1C), 44.7 (1C), 52.5 (1C), 104.8 (1C, C=C), 110.3 (1C, C=C), 121.9 (1C, C=C), 126.5 (1C, Ph), 128.4 (2C, Ph), 128.7 (2C, Ph), 135.5 (1C, Ph), 139.5 (1C, C=C), 147.5 (1C, C=C), 172.6 (1C, CO), 177.5 (1C, CO), 178.6 (1C, CO). HRMS *m/z* calcd for $C_{21}H_{19}N_6O_3$ $[M-H]^+$ 403.1519, found 403.1524.

2,7,10-Triamino-*N*^{2}-phenylacetyl-10-decarbamoylmitosene (14)

Synthesis from 21: Compound **21** (20 mg, 0.0494 mmol) was dissolved in 1.2 mL of pyridine at room temperature. Ammonium hydroxide (0.6 mL of a 30% solution) and triphenylphosphine (45 mg, 0.171 mmol) were added. The mixture was stirred at room temperature for 36 h. The purple resulting product was isolated by chromatography. (SiO_2: 7.5% MeOH, 2% NEt_3, 81.5% CH_2Cl_2). (18 mg, 96 % yield). *Synthesis from 13:* Compound **13** (15 mg, 0.0295 mmol) was dissolved in 20 mL of 80% hydrazine in ethanol. The mixture was stirred overnight at room temperature. Solvents were evaporated and the purple residue was purified by chromatography (SiO_2: 7.5% MeOH, 2% NEt_3, 81.5% CH_2Cl_2). (10 mg, 89% yield).^{1}H NMR (methanol-*d*$_4$) δ 1.83 (s, CH_3, 3H), 2.93 (dd, H_1, *J*=4.8, 16.3 Hz, 1H), 3.29 (dd, H_1, *J*=7.5, 16.3 Hz, 1H), 3.54 (s, CH_2, 2H), 4.12 and 4.16 (AB quartet, H_{10}, *J* =13.9 Hz, 2H), 4.12 (dd, H_3, *J*=3.5, 12.5 Hz, 1H), 4.51 (dd, H_3, *J*=7.5, 12.9 Hz, 1H), 4.96 (m, H_2, 1H), 7.30 (m, H_{ar}, 5 H). ^{13}C NMR ($CDCl_3$/methanol-*d*$_4$)δ 7.9 (1C, CH_3), 29.4 (1C), 35.7 (1C), 42.9 (1C), 52.6 (1C), 53.5 (1C), 107.1 (1C, C=C), 113.2 (1C, C=C), 122.4 (1C, C=C), 126.9 (1C, Ph), 128.6 (2C, Ph), 129.1 (2C, Ph), 129.4 (C, C=C), 134.8 (1C, Ph), 139.1 (1C, C=C), 145.6 (1C, C=C), 171.7 (1C, CO), 177.5 (1C, CO), 179.6 (1C, CO). HRMS *m/z* calcd for $C_{21}H_{23}N_4O_3$ $[M+H]^+$: 379.1770, found: 379.1751.

2,7,10-Triamino-10-decarbamoylmitosene (15)

Synthesis from 14: A solution of **14** (20 mg, 53 mmol) in MeOH (50 mL) and potassium phosphate buffer, (0.05M, pH 8.5, 100 mL) was treated with penicillin amidase (0.540 mL, 500 units). The resulting solution was gently stirred for 18 h at room temperature, when TLC analysis (5% MeOH in CH_2Cl_2) showed the disappearance of **14**. The mixture was partially concentrated to remove MeOH. The resulting aqueous solution was acidified with 1 M HCl and extracted with EtOAc-hexane (1:1, 2 x 40 mL) to remove traces of **14**. The aqueous phase was treated with saturated aqueous Na_2CO_3 until pH 10−11, then extracted with CH_2Cl_2 (3 x 40 mL). The combined extracts were dried and concentrated, to give **15** as a purple solid (12 mg, mmol, 87%). *Synthesis from (22):* Triphenylphosphine (45 mg, 0.17 mmol) was added to a solution of **22** (10 mg, 0.035 mmol) in pyridine (1.2 mL) and concentrated ammonia (0.6

mL). The reaction mixture was stirred for 36 h under Ar at room temperature. TLC analysis (1% concentrated NH_4OH, 7% MeOH, 92% CH_2Cl_2) showed the disappearance of **22**, and the formation of a single new compound. The reaction mixture was concentrated in vacuum, and the resulting residue was redissolved in water. The aqueous phase was washed with hexane, EtOAc and CH_2Cl_2 (2 x 40 mL each). The aqueous phase was reduced to 3 mL and eluted through a Sep-Pak cartridge with water using a gradient of 0% to 40% methanol in water. The eluting purple solution was collected and lyophilized to give **15** (7 mg, 0.027 mmol, 77%) as a purple solid. ^{1}H NMR (methanol-d_4) δ 1.83 (s, CH_3, 3H), 2.63 (dd, H_1, J=4.5, 16.3 Hz, 1H), 3.13 (dd, H_1, J=7.5, 16.3 Hz, 1H), 3.95 (dd, H_3, J=3.5, 12.5 Hz, 1H), 3.97 (s, H_{10}, 2H), 4.20 (m, H_2, 1H), 4.39 (dd, H_3, J=6.5, 12.9 Hz, 1H). HRMS *m/z* calcd for $C_3H_{17}N_4O_2$ $[M+H]^+$: 261.1346, found: 261.1348.

Synthesis of protected adduct 17

2-Fluoro-O^6-(2-p-nitrophenylethyl)-deoxyinosine[19] (**16**) (18.8 mg, 0.0448 mmol) was dissolved in dry DMF (40 μl). Compound **14** (17 mg, 0.0449 mmol) and diisopropylethylamine (10μl, 7.42 mg, 0.958 mmol) were added to the reaction mixture which was incubated at 45° for 18 h. The resulting crude material was diluted with water (100 μL) and lyophilized. The desired product was isolated by preparative thin layer chromatography (SiO_2: 40% acetone 60% EtOAc) to give 15 mg (43% yield) of **17**. ^{1}H NMR (methanol-d_4): δ 1.80 (s, CH_3, 3H), 2.34 (ddd, $H_{2'}$, J=3, 6.2, 13.4 Hz, 1H), 2.64 (dd, H_1, J=4.6, 16 Hz, 1H), 2.82 (ddd, $H_{2''}$, J=2, 6.2, 13.4 Hz, 1H), 3.13 (dd, H_1, J=7.1, 15.5 Hz, 1H), 3.19 (t, CH_2, J=4.6 Hz, 2H), 3.42 and 3.43 (AB quartet, CH_2, J =14 Hz, 2H), 3.67 (bd, $H_{5'}$, J =12.2 Hz, 1H) and 3.77 (bd, $H_{5''}$, J =8.5 Hz, 1H), 3.99 (d, H_3, J =4.6 Hz, 1H), 4.02 (d, $H_{4'}$, J =4.4 Hz, 1H), 4.39 (dd, H_3, J =7.1, 12.9 Hz, 1H), 4.52 (m, $H_{3'}$, 1H), 4.67 (s, CH_2, 2H), 4.75 (t, CH_2, J=6.6 Hz, 2H), 4.87 (m, H_2, 1H), 6.35 (t, $H_{1'}$, J=3.3 Hz, 1H), 7.20−7.27 (m, H_{ar}, 5 H), 7.52 (d, H_{ar}, J=8.5 Hz, 2H), 8.13 (d, H_{ar}, J=8.5 Hz, 2H), 8.25 (bs, 1H). HRMS *m/z* calcd for $C_{39}H_{40}N_9O_9$ $[M+ H]^+$: 778.2949, found: 778.2922.

Synthesis of 18

The N^2- and O^6-protected mitosene-nucleoside adduct **17** (6 mg, 0.00771 mmol) was dissolved in acetonitrile/DMF (100μL/200μL) and was treated with DBU (30 μL, 30.54 mg, 0.201 mmol). TLC showed the disapearance of all starting material after 1.5 h. Water was added to the sample (1 mL) and the solution was lyophylized. The desired product was isolated by preparative thin layer chromatography (SiO_2: 3% triethylamine: 15% MeOH: 82% CH_2Cl_2) to give 4 mg (82% yield) of **18** as a red solid. ^{1}H NMR (methanol-d_4): δ = 1.81 (s, CH_3, 3H), 2.40 (ddd, $H_{2'}$, J=3.5, 6.2, 13.5 Hz, 1H), 2.69 (m, $H_{2''}$, 1H), 2.83 (dd, H_1, J=4.5, 16 Hz, 1H), 3.19 (m, H_1, 1H), 3.67 (dd, $H_{5'}$, J =4.8, 12.8 Hz, 1H) and 3.75 (dd, $H_{5''}$, J =3.9, 8.1 Hz, 1H), 3.99 (m, $H_{4'}$, 1H), 4.02 (dd, H_3, J =4.8, 13.2 Hz, 1H), 4.45 (dd, H_3, J =7.5, 13.2 Hz, 1H), 4.51 (bm, $H_{3'}$, 1H), 4.58 and 4.64 (AB quartet, CH_2, J =15 Hz, 2H), 4.96 (m, H_2, 1H), 6.35 (t, $H_{1'}$, J=7.1 Hz, 1H), 7.20 −7.29 (m, H_{ar}, 5 H), 8.00 (s, H_8, 1H), 8.11 (s, H_8, 1H). HRMS *m/z* calcd for $C_{31}H_{33}N_8O_7$ $[M + H]^+$: 629.2472, found: 629.2485.

Synthesis of 8

The N^2-protected mitosene-nucleoside adduct **18** (4 mg, 0.00636 mmol) was dissolved in a mixture of methanol (0.6 mL) and a potassium phosphate buffer (1.8 mL, pH=8.5, M=0.05, KH_2PO_4). Penicillin amidase (37 units, 40 μL) was added and the reaction was incubated overnight at room temperature. The crude mixture was diluted with methanol (15 mL) and a white precipitate appeared which was discarded after centrifugation. The solution was evaporated and the resulting brown residue was redissolved in methanol. A second white precipitate appeared and was discarded as above. The methanol solution was then evaporated to dryness. The black residue was taken up in water and washed three times with chloroform.

The aqueous layer was collected and lyophilized to give **8** (3 mg, 92% yield) as a brown solid. The compound was found to be pure by HPLC and NMR analysis. ^{1}H NMR (pyridine-d_5-D_2O): δ = 1.53 (s, CH_3, 3H), 2.19 (ddd, $H_{2'}$, *J*=3.5, 6.2, 13.3 Hz, 1H), 2.38 (app quint, $H_{2''}$, *J*=6.9 Hz, 1H), 2.65 (dd, H_1, *J*=4, 16 Hz, 1H), 2.77 (dd, H_1, *J*=7.1, 16 Hz, 1H), 3.52 (dd, $H_{5'}$, *J* =4.5, 12 Hz, 1H) and 3.59 (dd, $H_{5''}$, *J* =3.9, 11.8 Hz, 1H), 3.68 (dd, H_3, *J* =4.7, 12.5 Hz, 1H), 3.82 (m, H_2, 1H), 3.96 (dd, H_3, *J* =6.8, 12.8 Hz, 1H), 4.02 (app quartet, $H_{4'}$, *J* =5.7 Hz, 1H), 4.13 and 4.15 (AB quartet, CH_2, *J* =15 Hz, 2H), 4.50 (app quint, $H_{3'}$, *J* =3.4 Hz, 1H), 6.23 (t, $H_{1'}$, *J*=6.8 Hz, 1H), 7.85 (s, H_8, 1H). HRMS *m/z* calcd for $C_{23}H_{27}N_8O_6$ $[M+H]^+$: 511.2054, found: 511.2049.

2,7-Diamino-10-decarbamoyl-10-azido-mitosene (22)

A solution of **21** (30 mg, 0.074 mmol) in MeOH (50 mL) and 0.05 M potassium phosphate buffer pH 8.5 (100 mL) was treated with penicillin amidase (0.540 mL, 500 units). The resulting solution was gently stirred for 18 h at room temperature, when TLC analysis (5% MeOH in CH_2Cl_2) showed the disappearance of **21**, and the formation of a single new compound. The mixture was partially concentrated to remove MeOH. The resulting aqueous solution was acidified with 1 M HCl and extracted with EtOAc-hexane (1:1, 2 x 40 mL) to remove traces of **21**. The aqueous phase was treated with saturated aqueous Na_2CO_3 to pH 10−11, then extracted with CH_2Cl_2 (3 x 40 mL). The combined extracts were dried and concentrated, to give **22** as a purple solid (20 mg, 0.0699 mmol, 94%). TLC and NMR analysis showed that the product was of high purity, and was used in the next step without further purification. ^{1}H NMR (methanol-d_4): δ 1.82 (s, CH_3, 3H), 2.62 (dd, H_1, *J*= 4.5, 16.1 Hz, 1H), 3.21 (dd, H_1, *J* = 6.9, 16.1 Hz, 1H), 3.96 (dd, H_3, *J*= 4.4, 12.9 Hz, 1H), 4.26 (m, H_2, 1H); 4.43 (dd, H_3, *J*= 6.5, 12.9 Hz, 1H), 4.45 and 4.50 (AB quartet, H_{10}, *J* = 14.0 Hz, 2H), 4.87 (br s, NH_2, 2H). ^{13}C NMR (methanol-d_4) δ 8.1 (1C, CH_3), 29.7 (1C), 33.7 (2C), 45.6 (1C), 55.8 (1C), 107.3 (1C, Ph), 110.7 (1C, Ph), 122.1 (1C, Ph), 128.7 (1C, Ph), 139.8 (1C, Ph), 145.5 (1C, Ph), 177.7 (1C, CO), 178.6 (1C, CO). HRMS *m/z* calcd for $C_{13}H_{15}N_6O_2$ $[M+H]^+$: 287.1251, found: 287.1250.

2,7-Diamino-N^2-(2-trimethylsilyl)ethoxycarbonyl-10-decarbamoyl-10-azidomitosene (23)

4-Nitrophenyl-2-(trimethylsilyl)ethyl carbonate (29 mg, 0.10 mmol) and *i*-Pr_2NEt (0.021 mL, 16 mg, 0.10 mmol) were added to a solution of **22** (16 mg, 0.056 mmol) in *i*-PrOH (0.70 mL). The reaction mixture was stirred for 3 h at room temperature. TLC analysis (5% MeOH in CH_2Cl_2) showed the disappearance of **23**, and the formation of a single new compound. The reaction was quenched by addition of 0.050 mL of concentrated aqueous ammonia. The resulting mixture was concentrated in vacuum, the residue was dissolved in EtOAc (25 mL) and washed with saturated Na_2CO_3 (5 x 10 mL) to remove 4-nitrophenol. The organic phase was dried and concentrated in vacuum. The residue was purified by flash chromatography using a gradient of 10% to 0% hexane in CH_2Cl_2 to give **23** (22 mg, 0.051 mmol, 91%) as a purple solid. ^{1}H NMR (methanol-d_4):δ 0.07 (s, TMS, 9H), 1.02 (t, CH_2Si, *J* = 8.2 Hz, 2H), 1.79 (s, 3H, CH_3), 2.80 (dd, H_1, *J*= 5.2, 16.3 Hz, 1H), 3.24 (dd, H_1, *J* = 7.8, 16.3 Hz, 1H), 4.04 (dd, H_3, *J*= 5.2, 12.9 Hz, 1H), 4.18 (t, CH_2O, *J* = 8.1 Hz, 2H), 4.45 (s, H_{10}, 2H), 4.47 (dd, H_3, *J*= 7.2, 12.9 Hz, 1H), 4.80 (m, H_2, 1H). ^{13}C NMR (methanol-d_4) δ −1.3 (3C, TMS), 8.1 (1C, CH_3), 17.8 (1C), 31.1 (1C), 45.6 (1C), 55.3 (1C), 54.1 (1C), 63.6 (1C), 107.3 (1C, Ph), 110.9 (1C, Ph), 122.2 (1C, Ph), 128.8 (1C, Ph), 136.7 (1C, Ph), 145.5 (1C, Ph), 155.9 (1C, CO), 174.4 (1C, CO), 178.6 (1C, CO). HRMS *m/z* calcd for $C^{19}H_{26}N_6O_4NaSi$ $[M+Na]^+$: 453.1677, found: 453.1681.

2,7,10-Triamino-N^2-(2-trimethylsilyl)ethoxycarbonyl-10-decarbamoylmitosene (24)

Triphenylphosphine (35 mg, 0.13 mmol) was added to a solution of **23** (8 mg, 0.018 mmol) in pyridine (1.0 mL) and concentrated aqueous ammonia (0.5 mL). The reaction mixture was

stirred for 18 h under Ar at room temperature. TLC analysis (1% concentrated NH_4OH, 7% MeOH, 92% CH_2Cl_2) showed the disappearance of **23**, and the formation of a single new compound. The reaction mixture was concentrated in vacuum, and the resulting residue was purified by flash chromatography using a gradient of 0 to 2% NH_4OH in MeOH/CH_2Cl_2 (7:100) to give **24** (7 mg, 0.017 mmol, 93%) as a purple solid. ^{1}H NMR (methanol-d_4): δ 0.07 (s, TMS, 9H), 1.07 (t, CH_2Si, J = 8.2 Hz, 2H), 1.80 (s, CH_3, 3H), 2.76 (dd, H_1, J= 5.1, 16.1 Hz, 1H), 3.21 (dd, H_1, J = 7.6, 16.1 Hz, 1H), 3.79 (s, H_{10}, 2H), 4.04 (dd, H_3, J= 5.0, 12.9 Hz, 1H), 4.18 (t, CH_2O, J = 8.1 Hz, 2H), 4.44 (dd, H_3, J= 7.0, 12.9 Hz, 1H), 4.78 (m, H_2, 1H). ^{13}C NMR (methanol-d_4) δ −2.9 (3C, TMS), 6.8 (1C, CH_3), 17.2 (1C), 29.1 (1C), 35.3 (1C), 55.4 (1C), 53.7 (1C), 62.7 (1C), 104.8 (1C, Ph), 114.6 (1C, Ph), 122.1 (1C, Ph), 129.2 (1C, Ph), 138.8 (1C, Ph), 147.4 (1C, Ph), 157.2 (1C, CO), 177.5 (1C, CO), 179.2 (1C, CO). HRMS *m/z* calcd for $C_{19}H_{29}N_4O_4Si$ $[M+H]^+$: 405.1952, found: 405.1950.

Synthesis of 5'-d(CTAGTG(18)TATCC) (20a)

The oligonucleotide 5'-d(CTAGTG(X)TATCC) (**19a**; X=2-fluoro-O^6-(2-trimethylsilylethyl)-deoxyinosine; 10 A_{260} units) was mixed in a vial with diisopropylethylamine (25 μL), DMSO (60 μL) and **14** (1 mg, 0.0026 mmol). The mixture was stirred at 42 °C for 72 h adding additional portions of **14** (0.5 mg) after 24 and 48 h. The reaction was stopped and the mixture was diluted with water (0.5 mL) then lyophilized. 1 mL of 5% acetic acid was added and the solution was stirred at room temperature for 3 h, followed by neutralization with 0.1 M NaOH to pH 7. The modified oligonucleotide (**20a**) was isolated by HPLC (6 A_{260} units, 60%) and characterized by MS (negative electrospray; deconvolution). *m/z* calcd for [M]: 3997.57, found: 3997.77.

Synthesis of 5'-d(GCTAGC(18)AGTCC) (20b)

Following the procedure described for **20a**, **20b** was prepared from oligonucleotide **19b** in 65% yield. It was characterized by MS (negative electrospray; deconvolution). *m/z* calcd for [M]: 4007.57, found: 4007.66.

Synthesis of 5'-d(CTAGTG(8)TATCC) (26)

The oligonucleotide 5'-d(CTAGTG(X)TATCC) (**19a**; X=2-fluoro-O^6-(2-trimethylsilylethyl)-deoxyinosine; 30 A_{260} units) was mixed in a vial with diethylamine (20 μL), DMSO (150 μL) and **24** (6 mg, 0.015 mmol). The mixture was stirred at room temperature. The reaction was monitored by HPLC after 24, 48 and 72 h. No starting material was observed after 72 h and the reaction was stopped. The mixture was diluted with 750 μL of water and 100 μL of 3 M NaOAc, pH=5. Ethanol (3 mL) was added and the mixture was left at −20 °C for 20 min. The suspension was centrifuged (13000 rpm for 15 min) and the supernatant was discarded. The brown residue was washed twice more with ethanol (750 μL). The crude modified oligonucleotide **25** (24 A_{260} units, 80 %) was dried in air and dissolved in a $ZnBr_2$ solution (300 μL, made as follow: 250 mg of $ZnBr_2$, 150 μL of nitromethane, 150 μL of isopropanol). The mixture was incubated at room temperature for 48 h and monitored by HPLC. The reaction was quenched with EDTA (12 mL of a 0.5 M solution) and the volume was reduced to half. A Sephadex G-25 gel filtration was performed. The void fraction containing the desired oligonucleotide **26** was liophylized and purified by HPLC (17 A_{260} units, 57 % overall yield). It was characterized by nuclease digestion and HRMS (negative electrospray; deconvolution). *m/z* calcd for [M]: 3877.7411, found: 3877.7318.

Enzymatic digestion of 26 to nucleosides

The oligonucleotide adduct **26** (1.3 A_{260} units) was dissolved in the following mixture: 700 μL of water, 200 μL of 0.5 M Tris-HCl pH 8.6 and 350 μL of 0.025M $MgCl_2$ and was incubated with snake venom phosphodiesterase (4 units) and alkaline phosphatase (4 units) at room

temperature for 8 h. The mixture was analyzed by reverse phase HPLC using the following gradient: 5−60% buffer B in buffer A in 75 min. (Buffer A: 0.3 M potassium phosphate, pH 5.8; buffer B: 70% buffer A, 30% acetonitrile). The modified nucleoside **8** was identified by comparison with an authentic sample based on retention time, co-injection and UV spectrum.

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1. Abbreviations: MC, mitomycin C; DAM, diaminomitosene[8]; ICL, interstrand cross-link; TAM, triaminomitosene[8]; TMSE, 2-trimetylsilylethyl.
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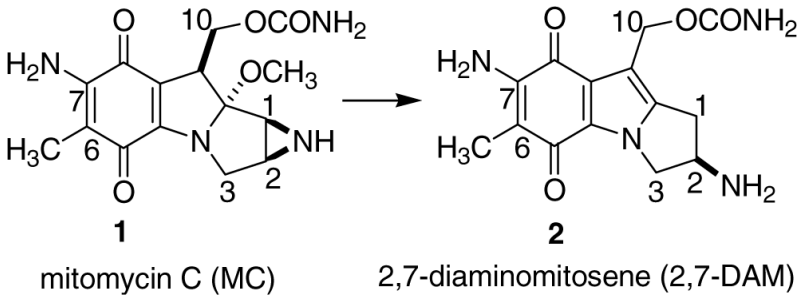
Supplementary Material

Refer to Web version on PubMed Central for supplementary material.



Scheme 1.
Reductive conversion of mitomycin C to 2,7-diamino-mitosene

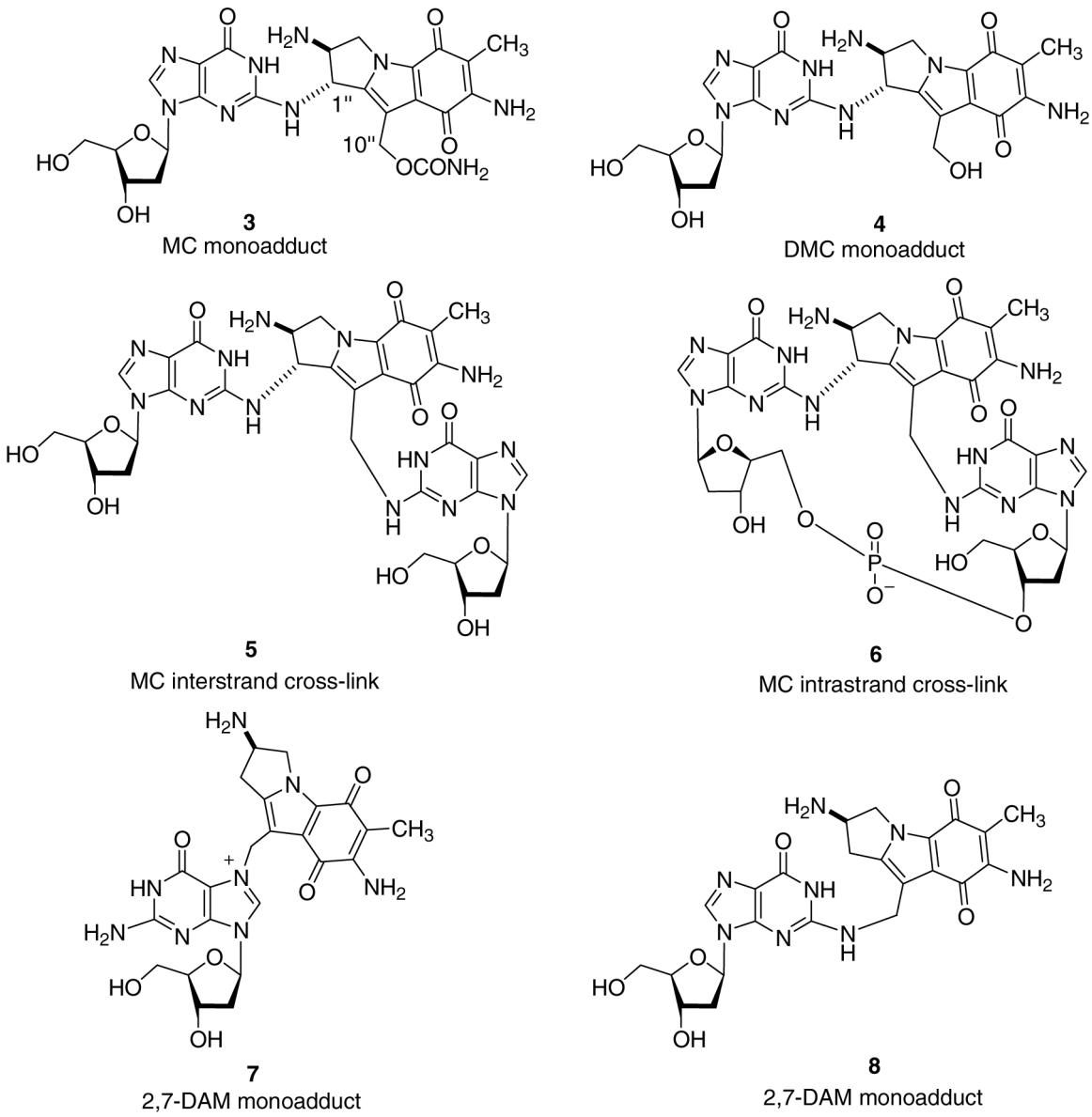
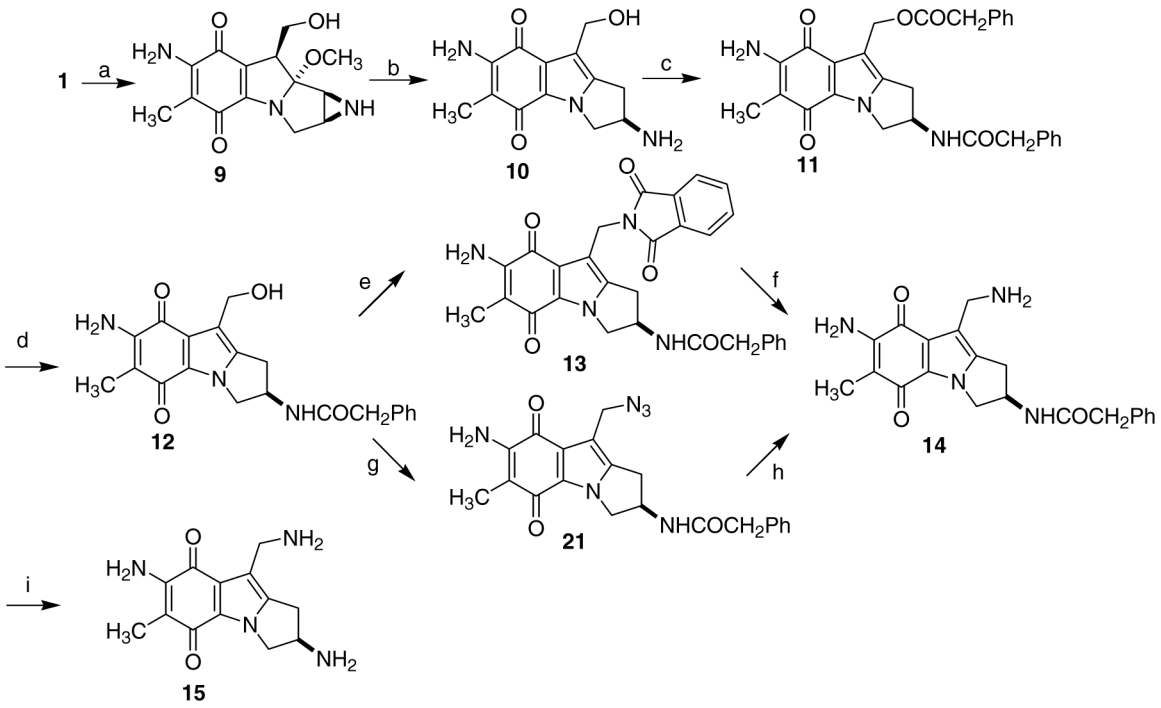


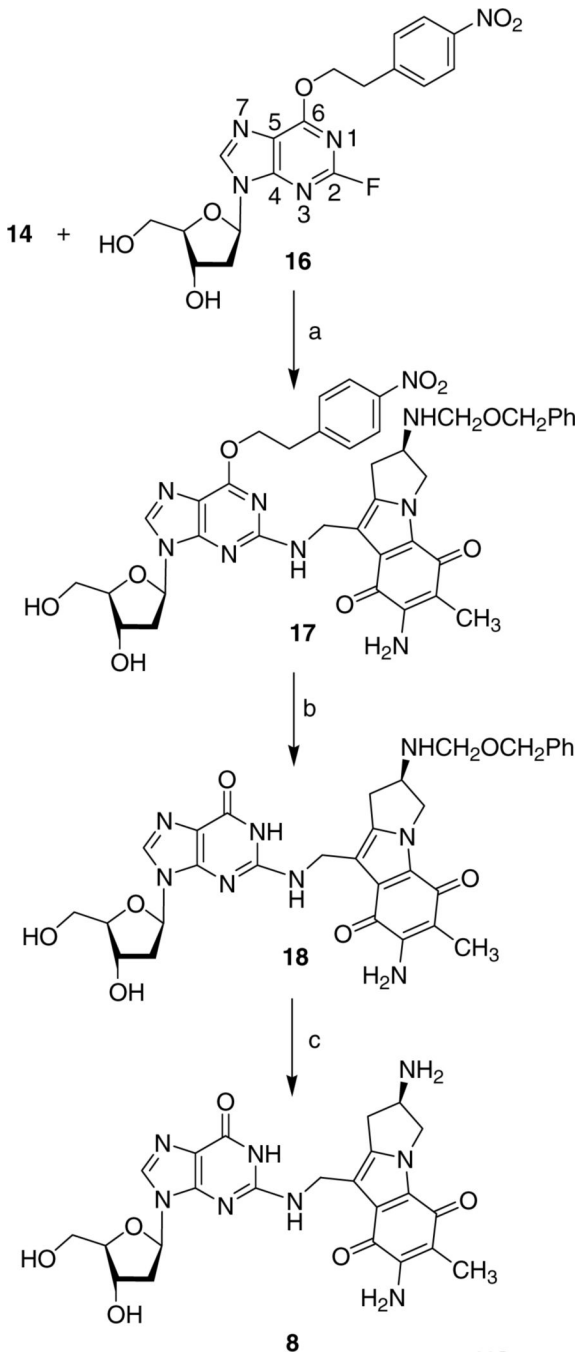
Chart 1.
The six major DNA adducts of mitomycin C.



Scheme 2.

Synthesis of 2,7,10-triamino-10-decarbamoylmitosene **15**

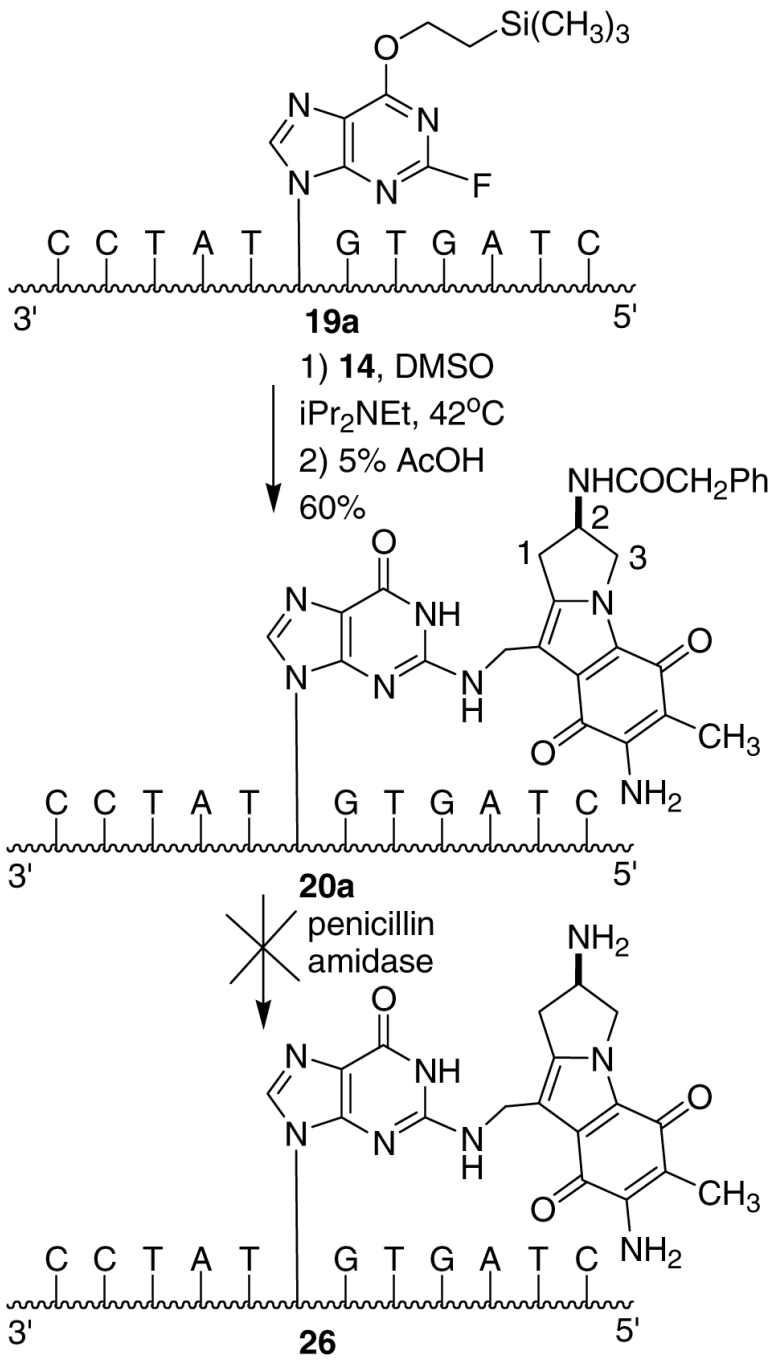
[a] Reagents: (a) CH_3ONa, toluene, 80%, (b) 5% Pd/C, MeOH, 75%, (c) phenylacetic anhydride, pyridine, 70%, (d) NH_3, MeOH, 90%, (e) phtalimide, DIAD, PPh_3, DMF, 50%, (f) hydrazine, EtOH, 80%, (g) methanesulfonyl chloride, pyridine followed by NaN_3, DMSO, 48%, (h) PPh_3, NH_4OH, THF/H_2O, 96% (i) penicillin amidase, potassium phosphate buffer, pH 8.5, aq. MeOH, 90%.



Scheme 3.

Synthesis of the nucleoside adduct **8**: Coupling of the N^2-protected triamino mitosene **14** to the 2-fluoro-O^6-(2-p-nitrophenylethyl)-deoxyinosine **16**

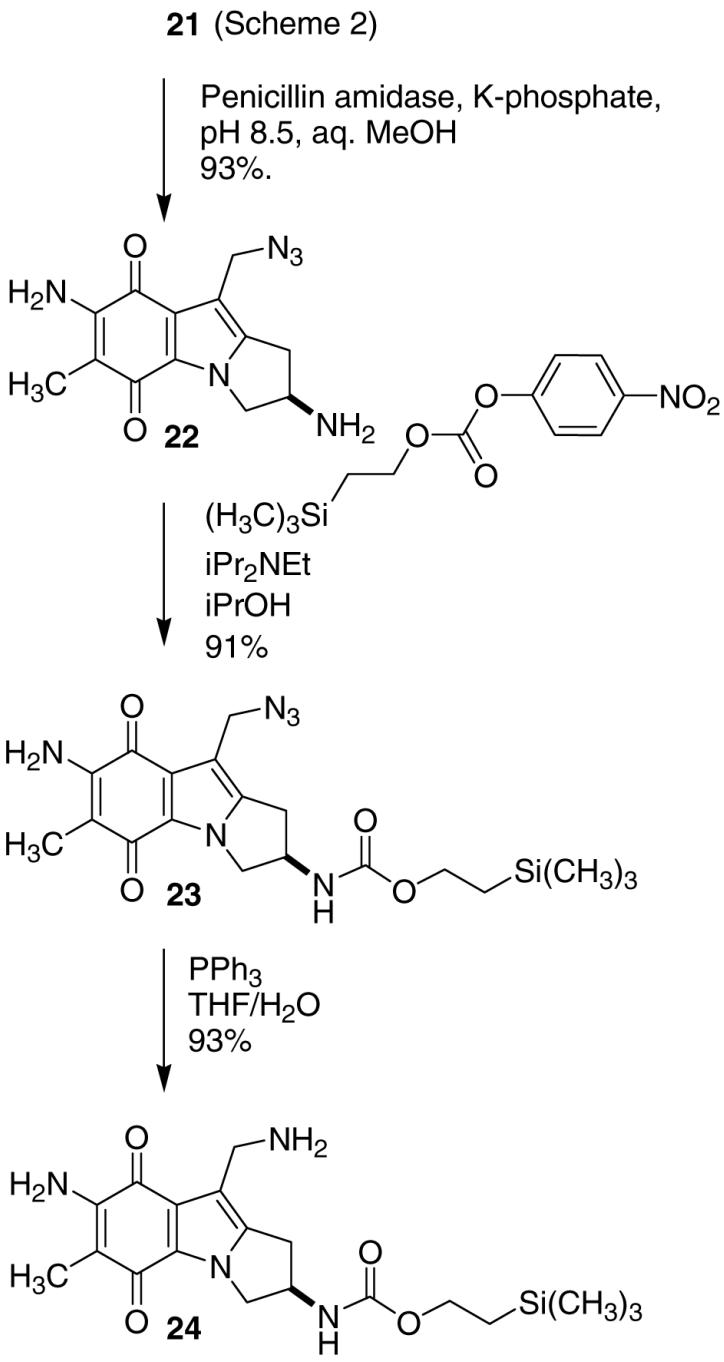
[a] Reagents: (a) Et_3N, DMF, 30%, (b) DBU, DMF/acetonitrile, 80%, (c) penicillin amidase, potassium phosphate buffer, pH 8.5, aq. MeOH, 85%.



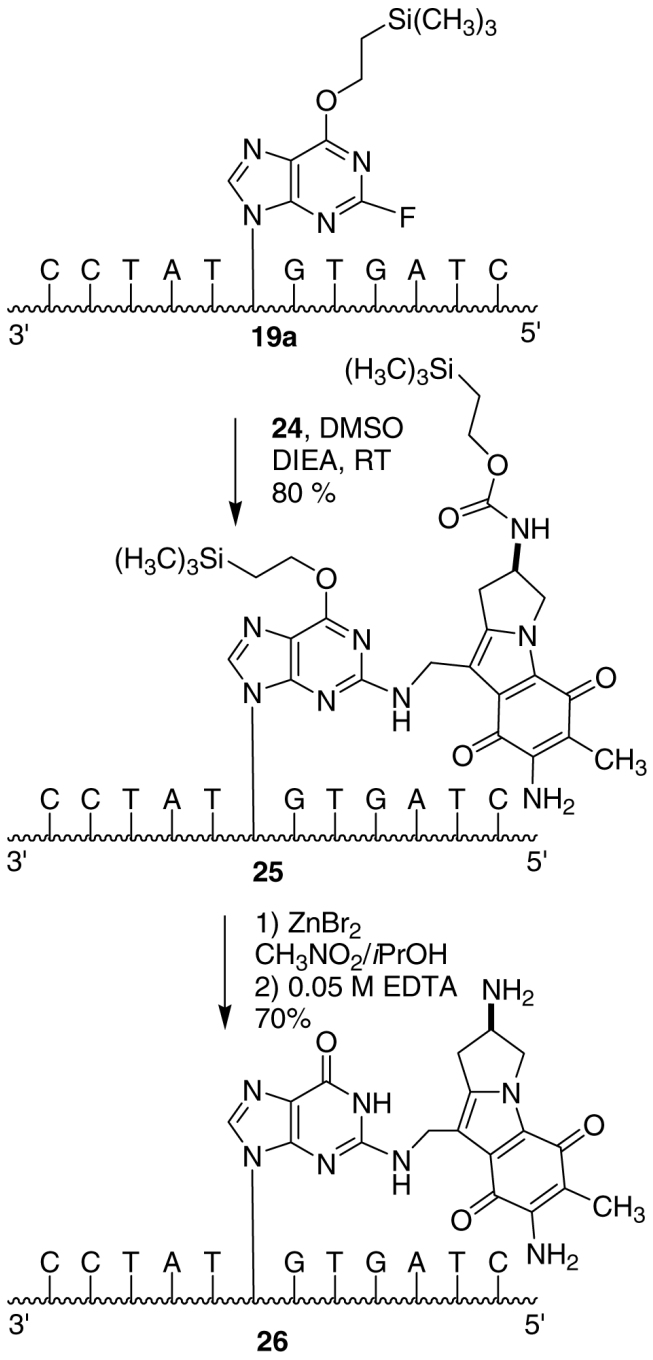
Scheme 4.

Coupling of **14** to the 12-mer oligonucleotide 5’-CTAGTGXTATCC-3’ (**19a**).[a]

[a] Coupling of **14** to 5’-GCT-AGC-XAG-TCC (**19b**) resulted in the corresponding product **20b** which, similarly to **20a**, was resistant to penicillin amidase.



Scheme 5.
Synthesis of N^2-protected 2,7,10-triaminomitosene (**24**).



Scheme 6.
Postoligomerization synthesis of the adduct **8**-substituted oligonucleotide (**26**).

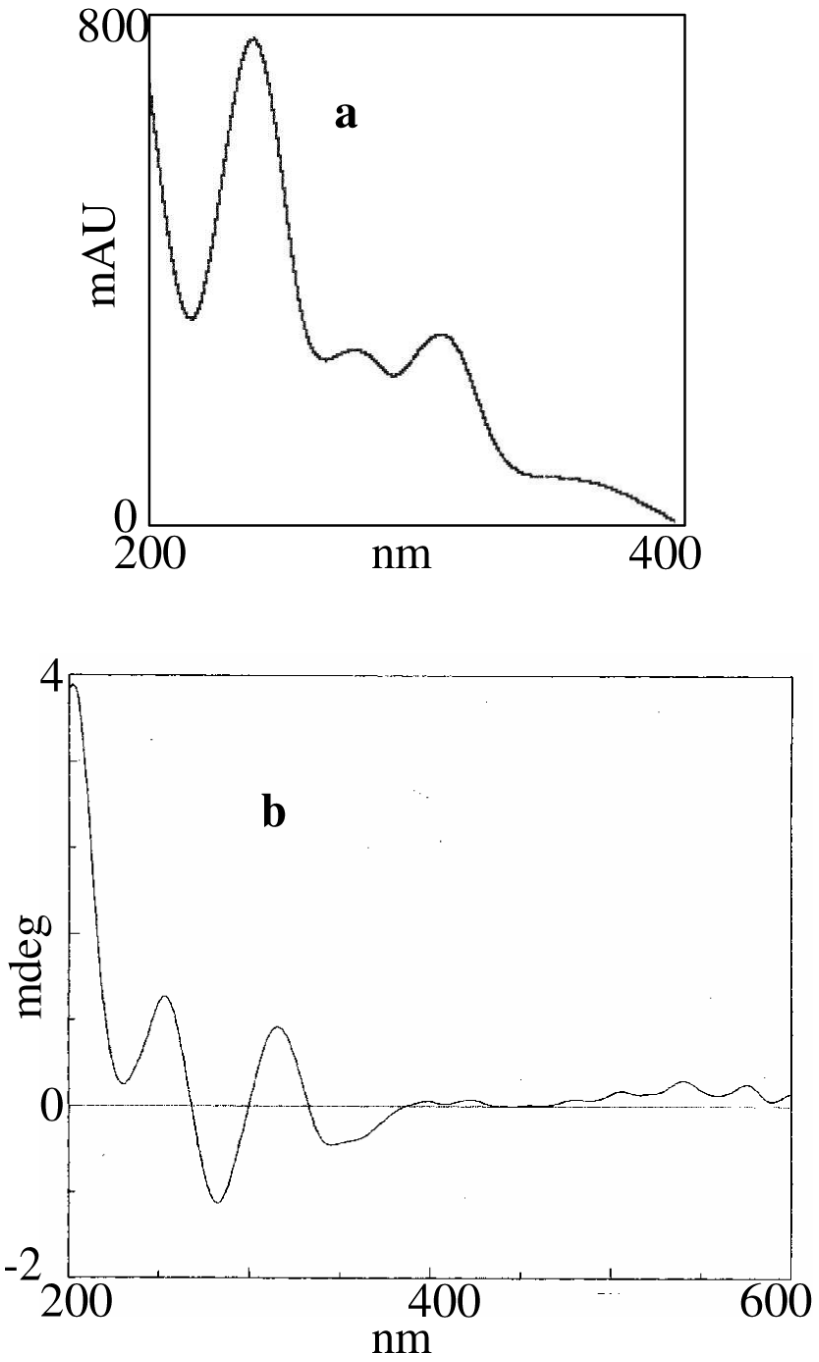


Figure 1.
Ultraviolet spectrum of adduct **8** (a) and circular dichroism spectrum of adduct **8** (b).

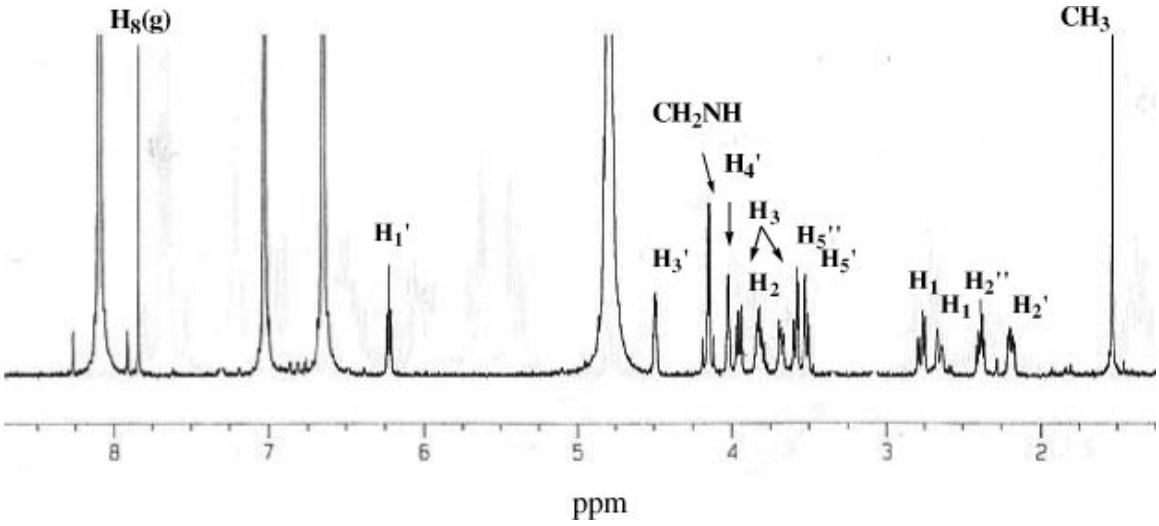


Figure 2.
^{1}H NMR spectrum of **8** in pyridine-d_5/D_2O. H_8(g), 8-proton of guanine.

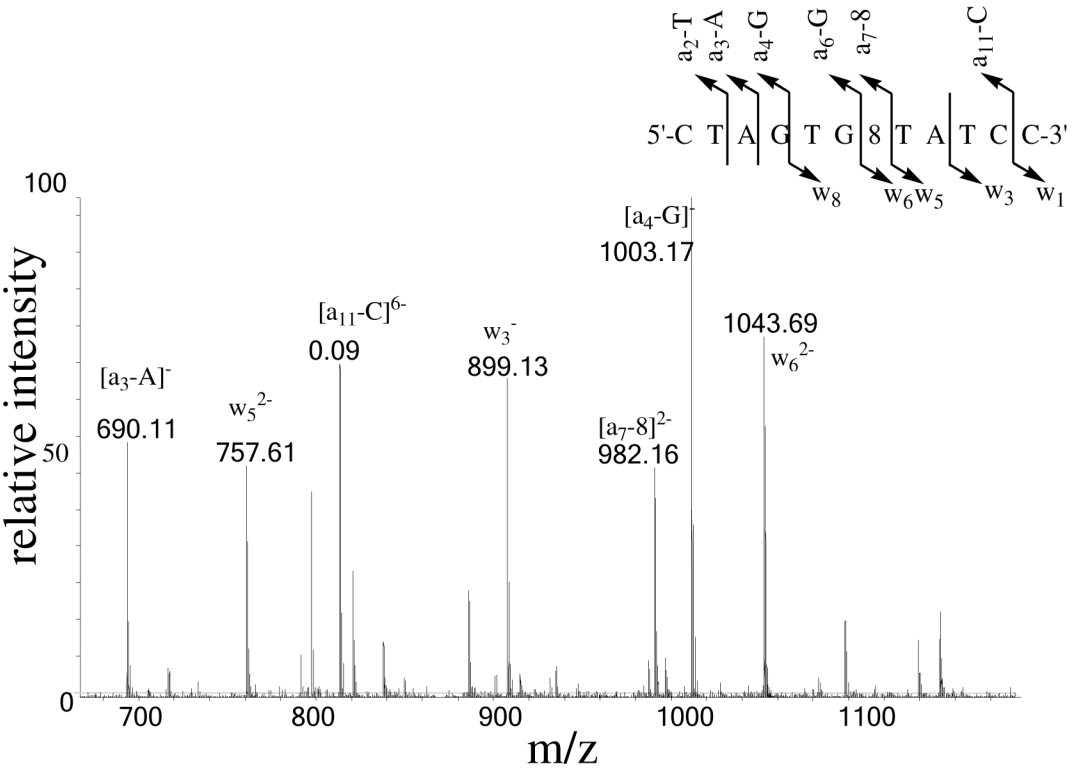


Figure 3.

MS-MS characterization of oligonucleotide **26**. Product ion spectrum of the $[M-3H]^{3-}$ ion (m/z=1292.24). For clarity purposes , w_1^- (306.04), w_8^{2-} (1360.80), $[a_2\text{-}T]^-$ (401.01) and $[a_6\text{-}G]^-$ (1636.18) are not represented.

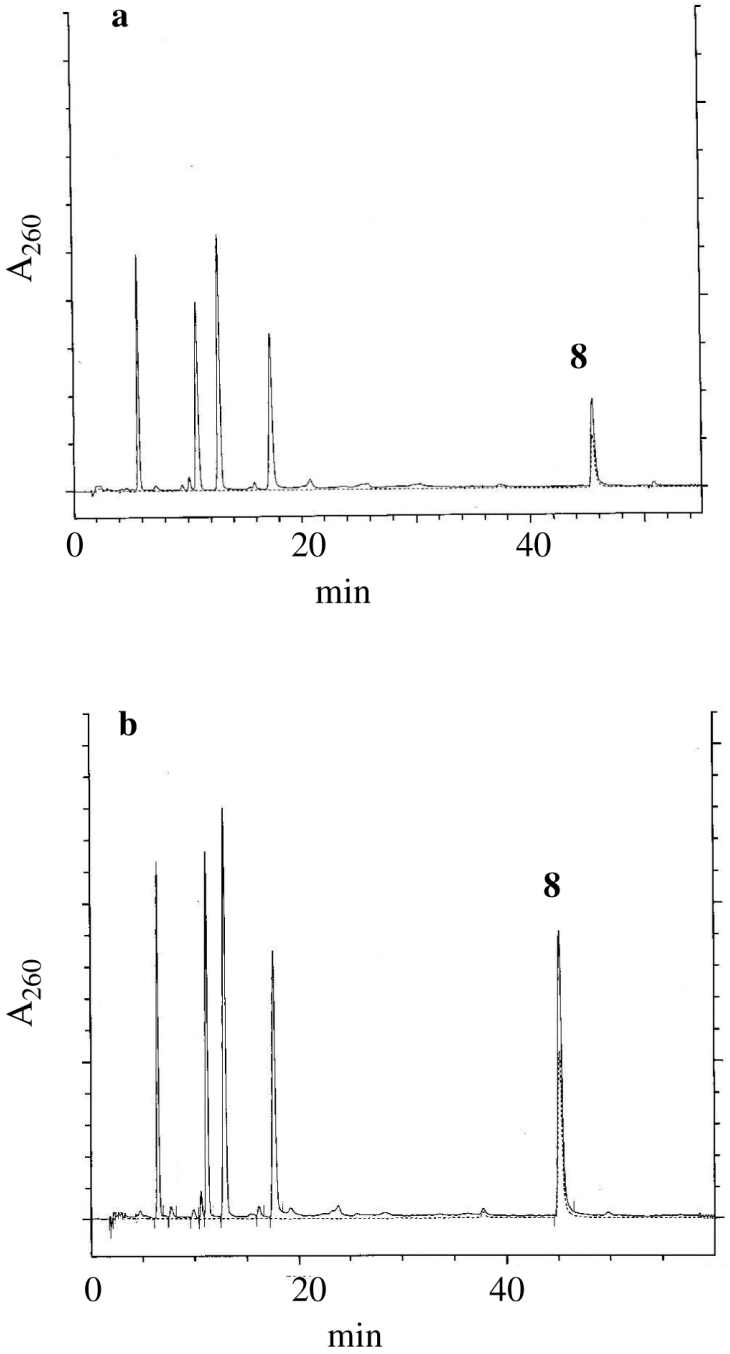


Figure 4.
(a) HPLC chromatogram of the digest of oligonucleotide **26**. (b) HPLC chromatogram of the co-elution of the digest with an authentic sample of **8**. (--- 260 nm, ----- 320 nm).