Journal of Computer-Aided Molecular Design, 6 (1992) 33–46
ESCOM

J-CAMD 145

Computer-aided molecular modeling and design of DNA-inserting molecules

Franca J.M. van der Klein-de Gunst, Jacques H. van Boom and Rob M.J. Liskamp*

Department of Chemistry, Gorlaeus Laboratories, University of Leiden, P.O. Box 9502, 2300 RA Leiden, The Netherlands

Received 6 May 1991
Accepted 26 August 1991

Key words: Diketopiperazine; Spacer; Bisinsertor; Bisintercalator; Molecular mechanics

SUMMARY

Intercalators are molecules capable of sliding *between* base pairs without disturbing the overall stacking pattern. In addition, there may exist molecules capable of inserting *into* a base pair thereby disrupting the hydrogen bonds and replacing them with new hydrogen bonds. A molecule probably capable of inserting, i.e., an insertor, is the diketopiperazine *cyclo*-[Gly-Gly] (**1**). A barbiturate (**2**), alloxan (**3**), a pyrimidine derivative (**4**) and a hydantoin (**5**) were also studied as possible insertors. Furthermore, molecules such as ethyleneurea (**6**), succinimide (**7**), as well as a malonamide derivative (**8**) and oxamide derivatives (**9–11**) were studied in order to investigate the arrangement and the number of hydrogen bonds necessary for insertion. Molecules **12–14** were designed and studied for their capacity to act as bisinsertors and/or bisintercalators. These molecules feature two diketopiperazine moieties which are connected via a diphenyl(thio)ether, i.e., **12** and **13**, or a bisphenol A spacer, i.e., **14**. The latter molecule (**14**) seems a promising candidate as a bisinsertor.

INTRODUCTION

Proteins that are capable of interacting with nucleic acids are essential in transcription and replication, which are among the most fundamental processes of the living cell. By interacting with DNA, these proteins are for example able to make specific genetic information accessible, while other nondesired information remains silent [1].

Two aspects of nucleic acid–protein interaction and the resulting events are especially intriguing: (1) the recognition of a specific sequence of bases and (2) unwinding or opening of the double helix.

In addition to the large and relatively complex nucleic-acid binding proteins, which are able to

* To whom correspondence should be addressed.

0920-654X/$ 5.00

realize these processes, nature provides us with small compounds also capable of interacting with DNA (vide infra).

Study of the details of interaction of these compounds with DNA has increased our understanding of the fundamental principles which underly the molecular basis of DNA recognition. Based on the availability of such information it is not unreasonable to attempt to design molecules capable of interacting with specific sequences in DNA. Such molecules could lead to mimetics of DNA-binding proteins, e.g., mimetics of restriction enzymes [2] and other nucleases [3], repressors and unwinding proteins. Furthermore these molecules could lead to new antiviral drugs or antitumor agents [4]. A number of small, naturally occurring compounds among which adriamycin (14-hydroxydaunomycin) are in fact used as antitumor agents.

The small naturally occurring compounds capable of interacting with DNA can be roughly divided in two classes: nonintercalating molecules such as the minor groove binders, e.g., netropsin [5,6] and distamycin [6] and the intercalating molecules.

Within the latter class the mono-intercalators actinomycin D [7,8] and the anthracycline antibiotic daunomycin [9] are found. Especially interesting is the subclass formed by the naturally occurring bisintercalators. Crystal structures of bisintercalators–oligonucleotide complexes are now available of the triostine [10,11] and echinomycin [12] complexes. In addition, crystal structures for the uncomplexed bisintercalators BBM-928A [13] and a synthetic analogue of triostin A, i.e., TANDEM [14], have been solved. These crystal structures provide valuable insights in the interactions, necessary for recognition of and intercalating in specific DNA sequences.

To a considerable extent the naturally occurring intercalators have served as a source of inspiration for the development of synthetic mono-, bis- and even trisintercalators. Well known intercalators are acridrines [15] and ethidium bromide [16], widely used in the field of biochemistry and molecular biology. A recent example of an intercalator consisting of connected aromatic rings was described by Wilson et al. [17]. Furthermore, DNA-intercalation by mono-intercalators can be assisted by attachment of a minor-groove binder [18] or by metal ions in crown–ether-linked intercalator derivatives [19].

Synthetic bisintercalators in which two phenanthridine molecules are linked together with a decamethylene chain or a diphenylether chain were described by Cory et al. [20]. Finally, the first DNA trisintercalator was described some years ago by Denny et al. [21].

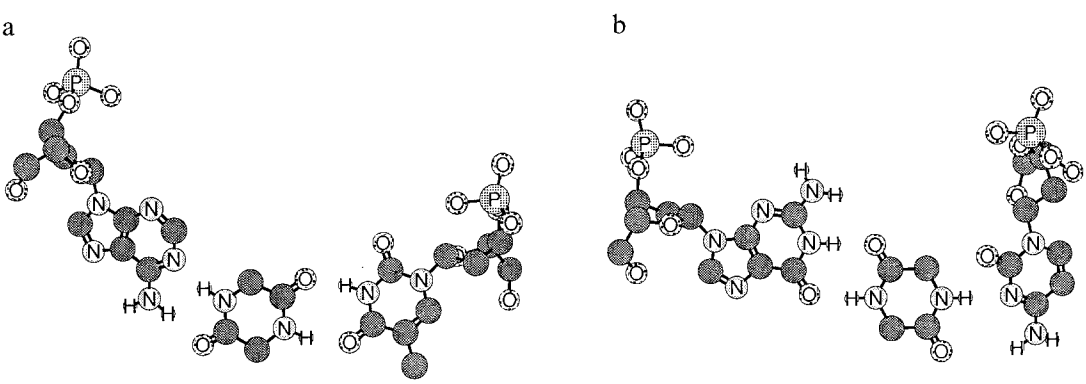


Fig. 1. Insertion of the diketopiperazine **1** in a (dA.dT) (a) and a (dC.dG) base pair (b).

Confronted with the fact that intercalators usually lead to an overall stiffening of the double helix [22] we raised the question whether it would also be possible to design molecules which are able to destabilize the double helix by insertion into the base pair and therefore separating the complementary bases. As to a way to achieve this we were inspired by the paper of Grafstein [23], who postulated for different reasons the interaction of a diketopiperazine with the bases of a base pair. We reasoned that the diketopiperazine ring and similar systems might be capable to insert into a base pair and consequently separate the two bases. This might lead to a destabilization and even a partial opening of the double helix. Therefore we embarked on modeling studies towards the computer-assisted molecular design of DNA-inserting molecules. A diketopiperazine molecule inserted into an A-T or a C-G base pair is shown in Fig. 1.

METHODS

The structures were built using the modeling program MacroModel [24] version 2.5 using a PS350 Evans & Sutherland graphics display linked to a VAX 3100 workstation. Molecular mechanics calculations were performed with Batchmin version 2.7 on a convex C 210 computer. The

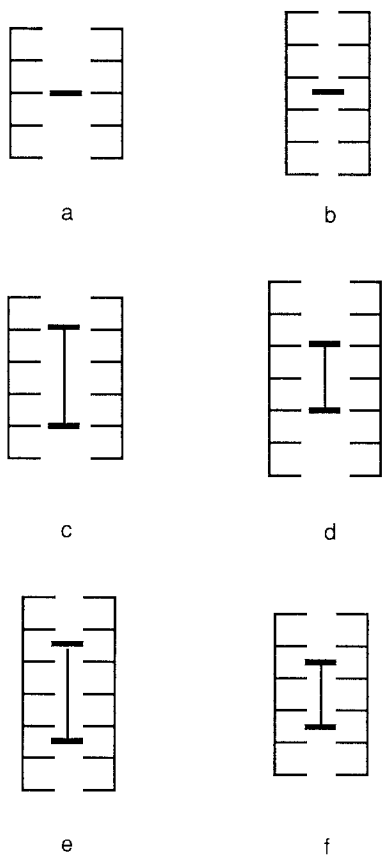


Fig. 2. Schematic representation of insertion (a) or intercalation (b) by compounds **1–11**; schematic representation of 1,4-insertion (c) or 1,3-insertion (d) by compounds **12–14**; schematic representation of a three-base-pair intercalation (e) or a two-base-pair intercalation (f) by compounds **12–14**.

minimization method used was Polak Ribière Conjugate Gradient (PRCG) with the United Atom Amber force field [25] in Batchmin until the RMS value was below 0.01 or until zero atom movement was detected. All minimizations were carried out in vacuo, each phosphate moiety had a sodium counter ion.

Modeling of the DNA-guest structures: Insertion and intercalation of a single guest molecule

The first step in modeling was to dock the guest molecule between a base pair (dA.dT or dC.dG) to get optimum hydrogen bonding between the base pair and the inserting molecule (Fig. 1). This base pair–guest complex was then superimposed on the middle base pair of a pentanucleotide DNA duplex, by superimposing nitrogen atoms N1 and N3 in the pyrimidine bases as well as nitrogen atoms N7 and N9 in the purine bases using a least–squares fit algorithm. Subsequently, the original base pair was removed and the bases of the base pair–guest complex were connected to the sugar phosphate backbone of the double helix followed by energy minimization. Insertion of a single guest molecule in a DNA duplex is schematically depicted in Fig. 2a.

For intercalation the guest molecule was docked between the two middle base pairs in a hexanucleotide DNA duplex followed by energy minimization, as is schematically depicted in Fig. 2b.

Insertion and intercalation of two guest molecules connected by a spacer

An analogous procedure was followed as for the insertion of a single molecule. For example, for 1,4-insertion a guest molecule was inserted into a separate base pair. This complex was superimposed on the second base pair of a hexanucleotide DNA duplex followed by superimposition of a base pair–guest complex on the 5th base pair. The original base pairs were removed and the new base pairs connected to the sugar-phosphate backbone. A spacer was then docked into either the minor or the major groove of the DNA helix and connected to both guest molecules followed by energy minimization. The stereocenters of molecules **12–14** (see Fig. 6) obtained in this way possessed the SS-configuration in most cases [26]. 1,4-Insertion and 1,3-insertion are schematically represented in Figs. 2c and 2d, respectively.

For intercalation the two guest molecules were docked between two base pairs of a heptanucleotide or hexanucleotide DNA duplex (Figs. 2e and 2f, respectively). The spacer was then docked in the minor or major groove and connected to the two intercalating molecules followed by energy minimization. The thus obtained molecules **12–14** possessed the SR (meso) configuration in most cases [26].

RESULTS AND DISCUSSION

As is shown in Fig. 1, a diketopiperazine can insert nicely into a (dA.dT) or (dC.dG) base pair. In addition to a diketopiperazine molecule, four other molecules were selected having an alternating arrangement of hydrogen bond donors and acceptors. Such an arrangement will allow the molecules **2–5** (see Fig. 3) to be inserted in a similar way as is the case with the diketopiperazine **1**. The heterocyclic ring in molecule **2** is reminiscent to the important class of the barbiturates [27]. Molecule **3** is the pyrimidine derivative alloxan, which destroys the capacity of the β-cells of the islets of Langerhans in the pancreas to produce insulin [28]. Molecule **4** is a pyrimidine derivative. The ring structure of molecule **5** is reminiscent to the class of the hydantoins, compounds possessing an anti-epileptic activity [29].

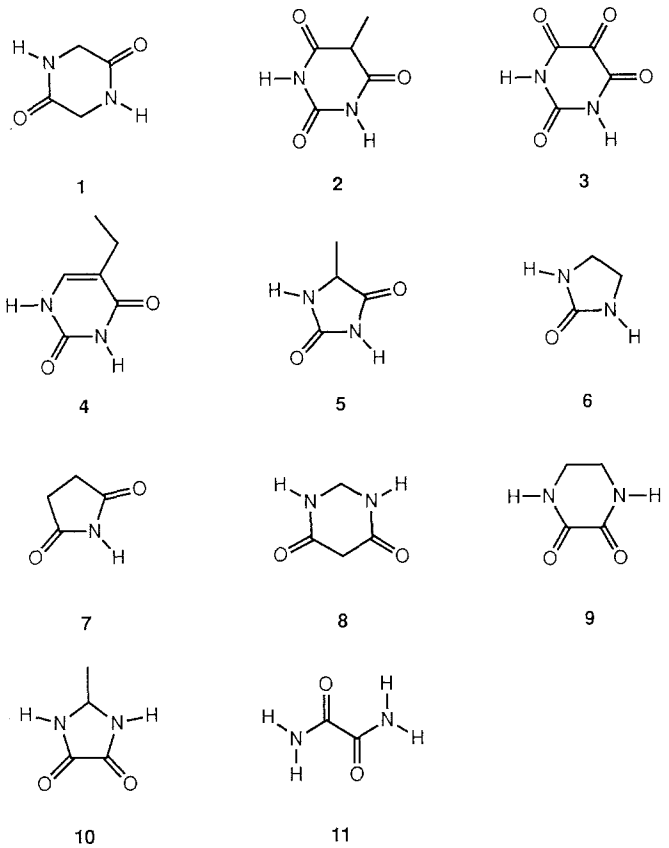


Fig. 3. Compounds used for modeling insertion and intercalation (Fig. 2) in DNA.

These molecules were inserted in a penta (dA.dT) DNA duplex. After minimization the structures remained nicely inserted in the base pair. The planar 6-membered rings **2**, **3** and **4** though, seemed to be a little bit too wide for insertion in a base pair as compared to both the boat-shaped and therefore less wide diketopiperazine **1** (Fig. 4a) and the 5-membered ring **5** (**2**, **3** and **4**: ca. 5.1 Å; as compared to **1** and **5**: ca. 4.4 Å). Consequently, the inserted guests **2**, **3** and **4** were slightly tilted, whereas the guests **1** (Fig. 4a) and **5** were not tilted. However, the calculated energy of all insertion complexes of molecules **1–5** was more favorable as compared to the energy of isolated guests and double-stranded DNA helix as is shown in Table 1.

In order to investigate if (a) alternating arrangement of hydrogen bond donors and acceptors as in **1** is necessary (b) two hydrogen bond donors and two acceptors are necessary (c) the hydrogen bond acceptors and donors have to be tied up in a ring structure, six other structures were inserted in a penta (dA.dT) sequence, i.e. **6–11**. The ethylene urea molecule **6** containing two hydrogen bond donors and one acceptor gave – after energy minimization – an insertion complex having a geometry similar to those obtained with the 6-membered ring structures **1–5**. In terms of energy this insertion complex was not favorable as compared to the insertion complexes of molecules **1–5** with two acceptors. In contradistinction to molecule **6** the imide molecule **7** containing two hydro-

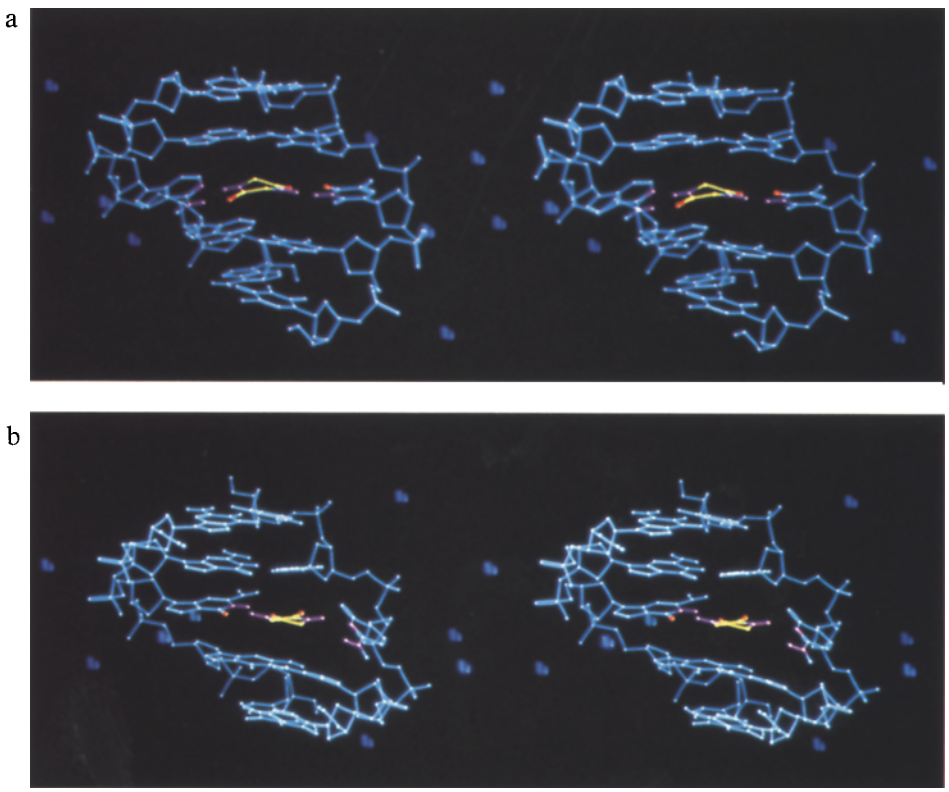


Fig. 4. Stereoviews of the insertion of the diketopiperazine **1** in penta (dA.dT) (a) and penta (dC.dG) (b).

TABLE I
MOLECULAR MECHANICS ENERGIES (kJ·mol^{-1}) RESULTING FROM INSERTION (Fig. 2a) OR INTERCALATION (Fig. 2b) OF COMPOUNDS **1–11**[a]

Compound	Insertion		Intercalation	
	In dA.dT	In dC.dG	In dA.dT	In dC.dG
1	−5.4	−49.7	−99.7	42.7
2	−26.3	−42.8	−42.6	
3	−17.6	−48.3	−56.7	
4	−54.2	−64.7	1.2	
5	−2.0	−51.5	−61.6	
6	32.0			
7	−58.5			
8	19.0			
9	−1.9			
10	9.5			
11	−58.5			

[a] The values represent the difference in molecular mechanics energy between the complex and the isolated guest and DNA molecule.

gen bond acceptors and one donor had a very favorable energy of insertion (Table 1). The geometry of insertion of molecule **7** also seemed to be better than that of molecule **6**. The favorable energy of insertion as well as the nice insertion geometry of **7** might be partially explained by the favorable electrostatic interaction of a sodium ion, that had moved inside the helix. Structures **8**–**10** having two hydrogen bond acceptors and two donors, though not in an alternating arrangement, formed – because of the arrangements of donors and acceptors – only three hydrogen bonds. Only molecule **8** displayed a nice insertion geometry, although the complex is less stable in terms of calculated energy.

The non-cyclic molecule oxamine **11** was capable of formation of four hydrogen bonds, but

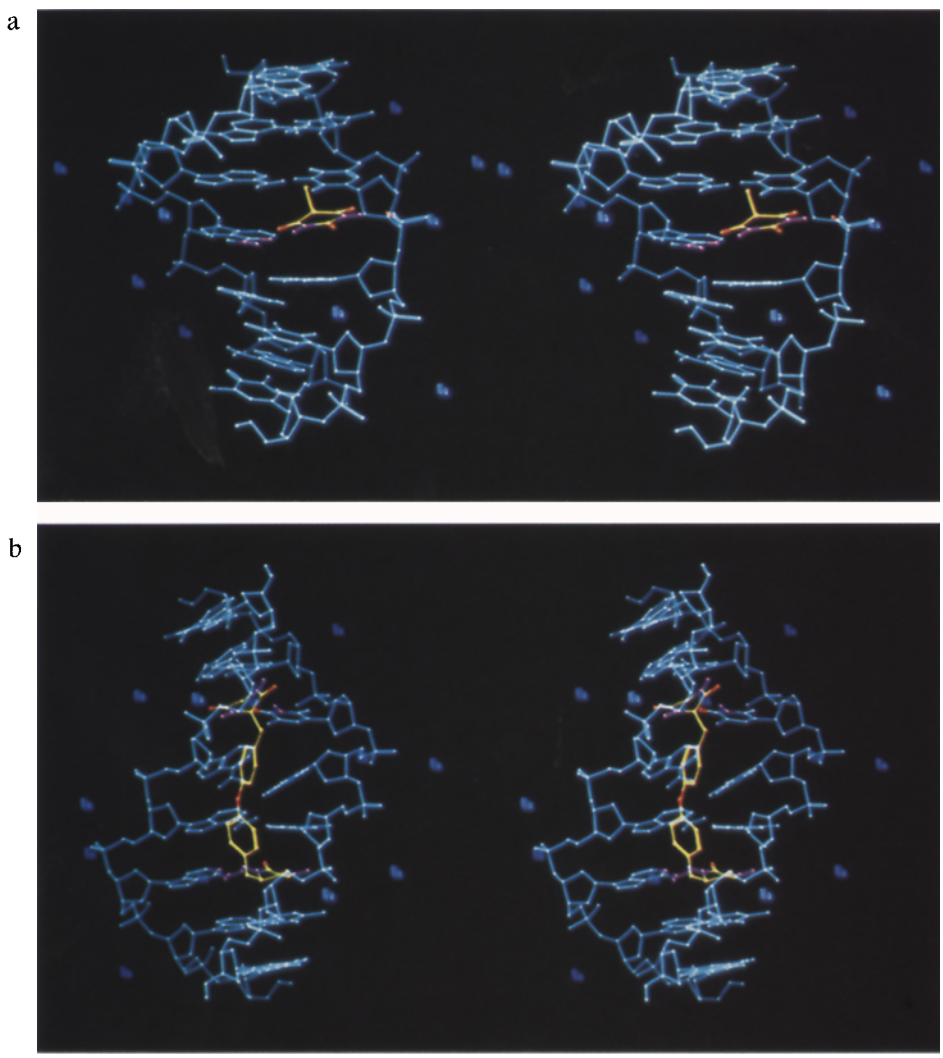


Fig. 5. Stereoviews of the intercalation of the barbiturate **2** in penta (dA.dT) (a) and base displacement by compound **14** (b) when a three-base-pair intercalation (Fig. 2e) was tried (see also Fig. 8).

gave rise to a distorted DNA helix, because of the linear, i.e. acyclic arrangement of hydrogen donors and acceptors.

In conclusion: a cyclic structure containing alternating hydrogen bonds and donors capable of forming four hydrogen bonds leads to an insertion complex of good geometry having a favorable molecular mechanics energy.

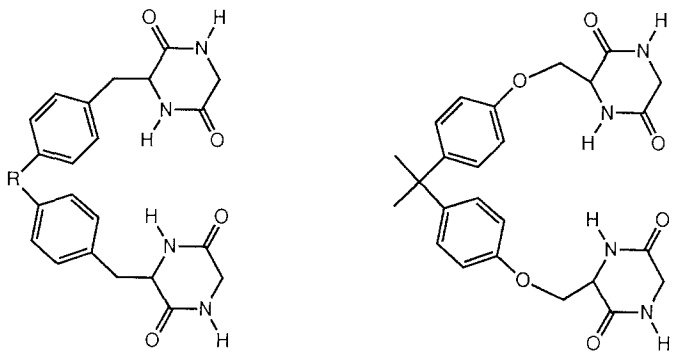
Molecules **1–5** were also inserted in a penta (dC.dG) sequence. The complexes of all these structures with the helix were stable but the geometries were poor, except for the diketopiperazine molecule **1**. Molecule **1** gave a reasonable geometry of the complex: parallel to adjoining bases and formation of four hydrogen bonds (Fig. 4b).

Molecules **1–5** can also be intercalated in the hexa (dA.dT) helix. The starting position of the intercalating molecule was between the 3rd and 4th base pair in the center of the helix (Fig. 2b). Structure **4** stayed in the center but this complex was not energetically favorable as compared to the isolated structures. The other structures (**1**, **2**, **3** and **5**) moved from the center of the helix to the position of the pyrimidine bases giving rise to a nice stacking with two pyrimidine bases. These complexes were stable (Table 1). The barbiturate **2** even took the place of an adjoining pyrimidine base, forming hydrogen bonds with the complementary purine base (Fig. 5a). The displaced pyrimidine forms hydrogen bonds with the purine below and the next pyrimidine base did not form any hydrogen bonds.

Finally, intercalating of diketopiperazine **1** in a penta (dC.dG) DNA double helix resulted in a reasonable geometry but poor energy as compared to the uncomplexed molecules. Thus, the diketopiperazine molecule **1** displayed the best insertion geometry in penta (dA.dT). In addition, insertion in both penta (dA.dT) and penta (dC.dG) was energetically favorable. Furthermore, the chemistry of diketopiperazines has been widely studied. Therefore this molecule was used in further investigations (vide infra). It is expected that if an organic molecule binds to DNA, a related molecule bearing two of these molecules will bind more strongly to DNA. This would be particularly true if the binding, i.e., inserting or intercalating units are placed at a certain distance of each other so that they can bind to DNA simultaneously. This concept has proven to be valuable in the design and synthesis of bisintercalators [20]. Although, e.g., polymethylene chains [30] or spermine [30] have been used as linkers or spacers for connecting the DNA-binding units, more rigid spacers seem more advantageous because it is likely that they position the DNA-binding units far better [20].

The diphenylether has been used previously as a spacer to connect two intercalating moieties, leading to a bisintercalator [20]. We used this spacer as well as the sulfur analog to connect two diketopiperazine moieties, possibly leading to bisinsertors **12** and **13** (Fig. 6). In addition, we incorporated 4,4′-isopropylidenediphenol (bisphenol A) as a spacer to connect two diketopiperazine moieties leading to the possible bisinsertor **14** (Fig. 6). The latter spacer – which is widely used as a cross-linker in polymer chemistry [31] – is even more rigid than diphenylethers because of the presence of the isopropylidene part. An additional advantage of these spacers is that they are commercially available and easily synthetically transformable.

A 1,4-base pair insertion in a hexa (dA.dT) helix, in which there are two base pairs between the base pair to be inserted, was first tried (Fig. 2c). After minimization all energies were very favorable for the complexes (Table 2). Bisinsertor **14** gave a nice geometry, both diketopiperazine units remained inserted in the base pairs and the spacer fitted nicely in the major (Fig. 7a) as well as in the minor groove (Fig. 7b), but in the major groove it turned *with* the handedness of the helix and



12 R=O
13 R=S

14

Fig. 6. Possible bisinsertors consisting of two diketopiperazine moieties and a diphenyloxy (**12**), a diphenylthio (**13**) or a 4,4′-isopropylidenediphenol (bisphenol A) (**14**) spacer.

TABLE 2
MOLECULAR MECHANICS ENERGIES (kJ·mol^{-1}) RESULTING FROM INSERTION (Figs. 2c and d) OR INTERCALATION (Figs. 2e and f) OF COMPOUNDS **12–14**[a]

Compound	1,4-Insertion in a (dA.dT) duplex		1,3-Insertion in a (dA.dT) duplex	
	Major groove	Minor groove	Major groove	Minor groove
12	−83.0	−123.7	−155.1	−164.3
13	−37.8	−45.2	−77.6	−34.3
14	−40.5	−90.6	−146.6	−140.6
Compound	3-Base-pair intercalation in a (dA.dT) duplex		2-Base-pair intercalation in a (dA.dT) duplex	
	Major groove	Minor groove	Major groove	Minor groove
12	−126.2	−83.2	−20.0	−105.5
13	−198.5	−173.4	−100.2	−67.8
14	−159.7	−99.4	−17.5	−124.6
Compound	1,4-Insertion in a (dC.dG) duplex			
	Major groove	Minor groove		
12	24.1	73.4		
13	41.0	21.1		
14	32.2	−0.5		

[a] The values represent the difference in molecular mechanics energy between the complex and the isolated guest and DNA molecule.

in the minor groove it turned slightly *against* the handedness of the helix. The diphenyloxy and diphenylthio moieties containing spacers present in bisinsertors **12** and **13**, respectively, seemed to be too short to span the two base pairs. Thus, when bisinsertors **12** and **13** were placed in the major groove one of the inserting units remained inserted but the other inserting unit was not present between the base pair anymore, but located in the major groove. If these bisinsertors were placed in the minor groove, again one of the inserting units remained between the two bases, but the other inserting unit took the place of the purine base of the base pair and pushed this base out of the center of the helix.

It was energetically more favorable if a bisinsertor was constructed having a spacer in the minor groove as compared to a spacer in the major groove (Table 2). Because the spacers in bisinsertors **12** and **13** seemed to be too short for 1,4-insertion (Fig. 2c), 1,3-insertion, in which there is only one base pair between the base pairs of insertion, was tried (Fig. 2d). The obtained geometries were not very good. In all cases the spacer now seemed to be too large for a reasonable insertion.

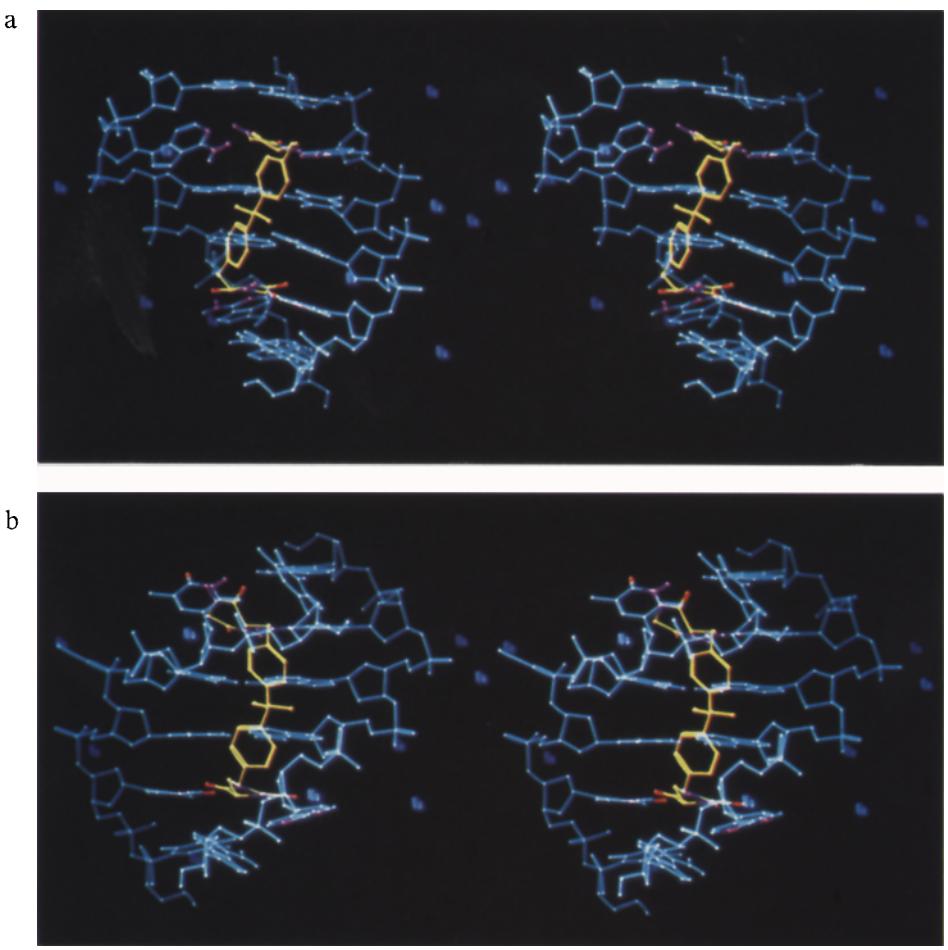


Fig. 7. (a) Stereoviews of bisinsertor **14** in the major groove of hexa (dA.dT) in a 1,4-insertion model (Fig. 2c); (b) bisinsertor **14** in the minor groove of (dA.dT) in a 1,4-insertion model (Fig. 2c).

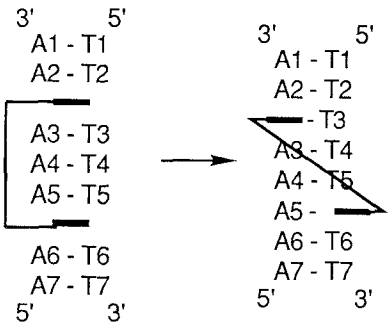


Fig. 8. Schematic representation of the base replacement by **12** and **13**.

The hydrogen bonds in the base pairs lying between the base pairs of insertion were also disrupted whereas in a 1,4-insertion the hydrogen bonds of base pairs between the inserted base pairs remained intact. The energy differences (Table 2), however, show that for **12** as well as **14** a 1,3-insertion is more favorable than a 1,4-insertion. A 1,3-insertion in the major groove is the most favorable insertion for **13**.

To investigate the possibility of intercalating we placed the two diketopiperazine moieties three base pairs apart (Fig. 2e). The spacer in compounds **12–14** was, however, too short to span three base pairs. If the diketopiperazine units were connected by the spacer in the major groove one of the intercalating units was pulled out into the major groove. Connecting the diketopiperazine units in the minor groove for compound **14** had the same effect: it pulled one of the intercalating units out of the center of the helix into the minor groove. Compounds **12** and **13** placed in the minor groove, behaved very differently. Both the intercalating units replaced a base of a base pair, but instead of pushing the replaced base out of the helix, recombination of base pairs followed as is shown in Fig. 5b and schematically represented in Fig. 8. The spacers were now spanning only two base pairs.

The spacers in **12–14** were clearly too short for a three-base-pair intercalation, therefore intercalation spanning two base pairs was also tried (Fig. 2f). Connecting the intercalating diketopiperazine units with the spacer in the major groove affording molecule **14**, led after minimization, to a situation in which one of the intercalating moieties was found in the major groove. When this modeling procedure was carried out with molecule **12** and **13** both diketopiperazine moieties remained intercalated, but one diketopiperazine moiety was slightly tilted.

Connecting the intercalating diketopiperazine units with the spacer in the minor groove affording molecule **14** approximated, after minimization, a situation which was described above for **12** and **13** for a three-base-pair intercalation in the minor groove. Compounds **12** and **13** placed in the minor groove pulled out one of the intercalating moieties into the minor groove.

A 1,4- and 1,3-insertion as well as three- and two-base-pair intercalation can, in principle, also be carried out employing a (dC.dG) sequence. However, as was outlined above intercalation of the diketopiperazine **1** in a (dC.dG) sequence was not energetically favorable (Table 1). Moreover, from modeling studies on insertion of **12–14** into a (dA.dT) sequence (vide supra) it was clear that 1,4-insertion gave better geometries than 1,3-insertion. Therefore, only 1,4-insertion (Fig. 2c) of **12–14** into a hexanucleotide (dC.dG) duplex was performed. It was found that the

energies of the complexes compared unfavorably to the sum of the strain energies of the isolated structures. In addition, it was found that, except for compound **14** which was placed in the major groove the geometries were poor too, in the sense that the inserting molecules tend to push a base out of the helix core into the major or the minor groove.

Finally, it seems appropriate to emphasize that the molecular mechanics energy differences listed in Table 2 should be interpreted with caution. Energy minimization is primarily used here to determine if a particular arrangement of the guest in the oligonucleotide duplex is possible at all. Evidently, this approach should be a starting point for more sophisticated considerations, which will have to take into account the more dynamic behavior of both the guest molecule and the oligonucleotide duplex. It is likely that an ensemble of conformations of the guest molecule around and including the global minimum will encounter a dynamic oligonucleotide duplex. However, although the DNA duplex is a dynamic entity, both molecular dynamics simulations and NMR data indicate that the B-DNA conformation remains essentially intact. Thus, it is justified to employ the B-DNA conformation in our considerations with respect to geometry and energy of insertion and intercalation of guest molecules. Another important aspect which should be dealt with in the near future is solvation. In order to obtain more realistic energy differences for deciding on insertion or intercalation, solvation energy terms of guest, oligonucleotide duplex as well as DNA–guest complex will have to be included in the summation of the energy compounds.

CONCLUSIONS

From the geometry of the obtained guest–DNA complexes it can be concluded that the diketopiperazine **1** is capable of inserting into a (dA.dT) or (dC.dG) duplex. Insertion of the hydantoin **5** led only to a nice geometry of the guest–DNA complex in a (dA.dT) duplex. However, in terms of the calculated molecular mechanics energy difference between the complex and isolated molecules, insertion of compounds **1–5** is possible in both a (dA.dT) and (dC.dG) duplex. Insertion of these compounds in the latter duplex is more favorable. Using the criterion of a favorable calculated energy of the complex, compounds **1–5**, with the exception of the pyrimidine derivative **4**, are also capable of intercalating in a (dA.dT) duplex.

From a synthetic point of view as well as from geometry considerations, there is a preference to use the diketopiperazine **1** and derivatives, in further experimental studies of the nature of guest–DNA interactions, i.e. insertion or intercalation. This does not mean that one should exclude the possibility that the other compounds having a particular arrangement of hydrogen bond donors and acceptors will not be capable of insertion or intercalation. Therefore this uncertainty will have to be taken into account in further synthetic studies and studies of the nature of guest–DNA interactions.

Based on the mono-insertion and intercalation modeling studies, compounds **12–14** were constructed having two diketopiperazine moieties connected by a spacer. It was shown that bisinsertion and bisintercalation is possible for compounds **12–14** in terms of energy. The 1,4-insertion of **14** in the major groove as well as the minor groove showed also a good geometry of the guest–DNA complex. Furthermore, in the guest–DNA complexes, obtained with molecules **12** and **13** after a three-base-pair intercalation, a base had been displaced in each of the opposing strands. In conclusion, in our opinion it is certainly worthwhile to investigate the validity of the predictions from this modeling study and earlier postulations [23] in further studies. Special emphasis will

have to be placed on the conformational behavior of the inserting or intercalating molecule and on calculating the solvation energies of guest, DNA duplex and guest–DNA complex. The results of these studies, which are in progress, will tell us whether it is possible to design, from first principles, molecules capable of interacting with biomacromolecules such as DNA.

REFERENCES

1 a. For reviews of DNA-binding proteins see e.g. Takeda, Y., Ohlendorf, D.H., Anderson, W.F. and Matthews, B.W., Science, 221 (1983) 1020.
b. Ollis, D.L. and White, S.W., Chem. Rev., 87 (1987) 981.
c. Brennan, R.G. and Matthews, B.W., Trends Biochem. Sci., (1989) 286.
d. Pabo, C.O. and Sauer, R.T., Annu. Rev. Biochem., 53 (1984) 293.
e. Matson, S.W. and Kaiser-Rogers, K.A., Annu. Rev. Biochem., 59 (1990) 289.
f. Harrison, S.C. and Aggarwal, A.K., Annu. Rev. Biochem., 59 (1990) 933.
g. Ohlendorf, D.H. and Matthews, B.W., Annu. Rev. Biophys. Bioeng., 12 (1983) 259.
2 a. Taylor, J.S., Schultz, P.G. and Dervan, P.B., Tetrahedron, 40 (1984) 457.
b. Schultz, P.G. and Dervan, P.B., Proc. Natl. Acad. Sci. U.S.A., 80 (1983) 6834.
c. Schultz, P.G., Taylor, J.S. and Dervan, P.B., J. Am. Chem. Soc., 104 (1982) 6861.
d. Schultz, P.G. and Dervan, P.B., J. Am. Chem. Soc., 105 (1983) 7748.
3 For a review on chemical nucleases see Sigman, D.S. and Chen, C.B., Annu. Rev. Biochem., 59 (1990) 207.
4 a. See e.g. Fico, R.M., Chen, T.-K. and Canellakis, E.S., Science, 198 (1977) 53.
b. Cain, B.F., Baguley, B.C. and Denny, W.A., J. Med. Chem., 21 (1978) 658.
5 a. Kopka, M.L., Yoon, C., Goodsell, D., Pjura, P. and Dickerson, R.E., J. Mol. Biol., 183 (1985) 553.
b. Kopka, M.L., Yoon, C., Goodsell, D., Pjura, P. and Dickerson, R.E., Proc. Natl. Acad. Sci. U.S.A., 82 (1985) 1376.
6 a. Zimmer, C., In Cohn, N.E. (Ed.) Progress in Nucleic Acids Research and Molecular Biology, Vol. 15, Academic Press, New York, 1975, pp. 285–318.
b. Gursky, G.V., Zasedatelev, A.S., Zhune, A.L., Khorlin, A.A., Grokhovsky, S.L., Streltsov, S.A., Surovaya, A.N., Nikitin, S.M., Krylov, A.S., Retchinsky, V.O., Mikhailov, M.V., Beabealashvili, R.S. and Gottikh, B.P., Cold Spring Harbor Symp. Quant. Biol., 47 (1982) 367.
7 Takusagawa, F., Dabrow, M., Neidle, S. and Berman, H.M., Nature, 296 (1982) 466.
8 a. Jain, S.C. and Sobell, H.M., J. Mol. Biol., 68 (1972) 1.
b. Sobell, H.M. and Jain, S.C., J. Mol. Biol., 68 (1972) 21.
9 Quigley, G.J., Wang, A.H.-J., Ugettho, G., van der Marel, G., van Boom, J.H. and Rich, A., Proc. Natl. Acad. Sci. U.S.A., 77 (1980) 7204.
10 Wang, A.H.-J., Ugettho, G., Quigley, G.J., Hakoshima, T., van der Marel, G.A., van Boom, J.H. and Rich, A., Science, 225 (1984) 1115.
11 Quigley, G.J., Ugettho, G., van der Marel, G.A., van Boom, J.H., Wang, A.H.-J. and Rich, A., Science, 232 (1986) 1255.
12 Ugettho, G., Wang, A.H.-J., Quigley, G.J., van der Marel, G.A., van Boom, J.H. and Rich, A., Nucleic Acids Res., 13 (1985) 2305.
13 Arnold, E. and Clardy, J., J. Am. Chem. Soc., 103 (1981) 1243.
14 Hossain, M.B., van der Helm, D., Olsen, R.K., Jones, P.G., Sheldrick, G.M., Egert, E., Kennard, O., Waring, M.J. and Viswamitra, M.A., J. Am. Chem. Soc., 104 (1982) 3401.
15 Lerman, L.S., J. Mol. Biol., 3 (1961) 18.
16 a. Waring, M.J., J. Mol. Biol., 13 (1965) 269.
b. LePecq, J.B. and Paoletti, C., J. Mol. Biol., 27 (1967) 87.
17 Wilson, W.D., Strekowski, L., Tanious, F.A., Watson, R.A., Morkrosz, J.L., Strekowska, A., Webster, G.D. and Neidle, S., J. Am. Chem. Soc., 110 (1988) 8292.
18 References 51 and 52 cited in Dervan, P.B., Science, 232 (1986) 464.
19 Fukuda, R., Takenaka, S. and Tagaki, M., J. Chem. Soc. Chem. Commun., (1990) 1028.
20 Cory, M., McKee, D.D., Kagan, J., Henry, D.W. and Miller, J.A., J. Am. Chem. Soc., 107 (1985) 2528.

21 Atwell, G.J., Leupin, W., Twigden, S.J. and Denny, W.A., J. Am. Chem. Soc., 105 (1983) 2913.
22 Saenger, W., Principles of Nucleic Acid Structure, Springer-Verlag, New York, 1983, p. 350.
23 Grafstein, D., J. Theor. Biol., 105 (1983) 157.
24 Mohamadi, F., Richards, N.G.J., Guida, W.C., Liskamp, R., Lipton, M., Caufield, G., Chang, G., Hendrickson, T. and Still, W.C., J. Comp. Chem., 11 (1990) 440.
25 a. Weiner, S.J., Kollman, P.A., Case, D.A., Singh, U.C., Ghio, C., Alagona, G., Profeta, S. and Weiner, P., J. Am. Chem. Soc., 106 (1984) 765.
b. Weiner, S.J., Kollman, P.A., Nguyen, D.T. and Case, D.A., J. Comp. Chem., 7 (1986) 230.
26 A study describing possible relations between the stereochemistry of **12–14** and interaction with DNA will be reported in due course.
27 Vida, J.A., In Wolff, M.E. (Ed.) Burger's Medicinal Chemistry, Part III, Wiley-Interscience, New York, 1981, pp. 787–828.
28 Lehninger, A.L., Biochemistry, Worth Publishers, New York, 1972, p. 502.
29 Isaacson, E.I. and Delgado, J.N., In Wolff, M.E. (Ed.) Burger's Medicinal Chemistry, Part III, Wiley-Interscience, New York, 1981, pp. 829–858.
30 See References 9 and 12, respectively, in Reference 20.
31 For a review see e.g. Chem. Eng. News., 51 (1973) 5.