Cannabimimetic Activity of Novel Enantiomeric, Benzofuran Cannabinoids

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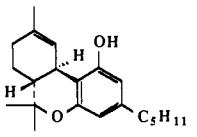
The synthesis of the (2*R*,3*R*,4*S*,6*R*)-**7**/(2*S*,3*S*,4*R*,6*S*)-**8** enantiomeric pair of benzofuran cannabinoids is reported together with the ^1H and ^{13}C NMR spectral parameters. In benzofuran **8** the configurational arrangement of ligated groups at the stereogenic C(3) atom (through which the terpene moiety is connected to the aromatic ring) is very similar to that of the corresponding atom in natural (3*R*,4*R*)-Δ^1-tetrahydrocannabinol (Δ^1-THC), although their respective Cahn–Ingold–Prelog descriptors are different. In drug-discrimination tests in pigeons and rats, benzofuran **8** is as active as Δ^1-THC; in the mouse ring test compound **8** is more active than Δ^6-THC. Enantiomer **7** is considerably less active than enantiomer **8** in both tests. These results can be explained by the fact that both **7** and **8** have a dimethylheptyl side chain (which is known to enhance cannabimimetic activity) and that Δ^1-THC and benzofuran **8** have closely related conformations, as determined by molecular mechanics.

We recently reported the synthesis and the psychotropic (cannabimimetic) action of the 1,1-dimethylheptyl homologues of the enantiomers of 7-hydroxy-Δ^6-tetrahydrocannabinol (**1** and **2**) (see Schemes I and II).[1] The 3*R*,4*R* enantiomer (**1**) is a very potent cannabimimetic, ca. 70–80 times more active than natural (3*R*,4*R*)-Δ^1-tetrahydrocannabinol (Δ^1-THC) (**3**) when tested in discrimination



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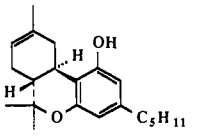

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tests in pigeons and rats and ca. 80–90 times more active than Δ^6-tetrahydrocannabinol (Δ^6-THC) (**4**) when tested in the rotarod or ring tests in rats or mice, respectively. By contrast, the 3*S*,4*S* enantiomer (**2**) was inactive in these tests when tested in doses up to several thousand times the ED_{50} of **1**.

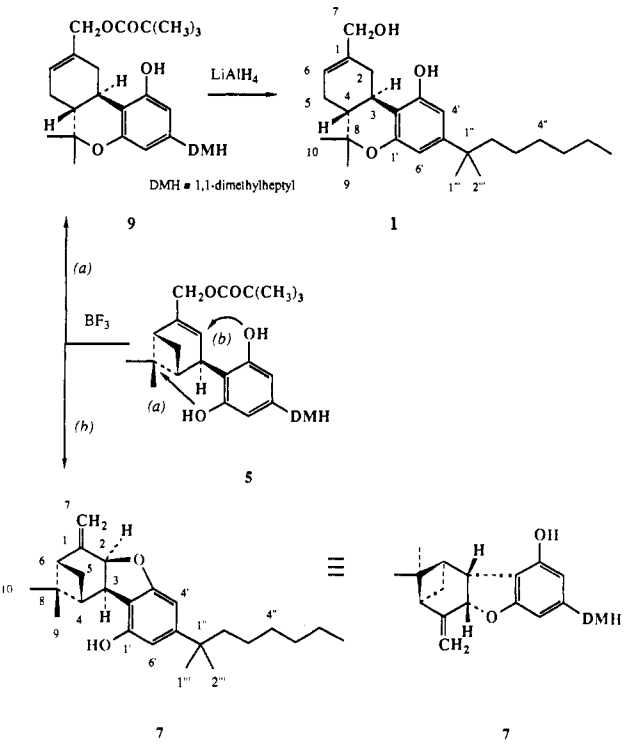
The synthesis of **1** and **2** involve in their penultimate step a ring opening of the cyclobutane ring in the intermediates (2*R*,3*R*,6*R*)-**5** and (2*S*,3*S*,6*S*)-**6**, respectively.[2] Crystalline side products obtained in these syntheses are benzofurans **7** and **8**, which are formed by ring closure of one of the phenolic hydroxyl groups with concomitant elimination of the ester grouping.

The olefinic double bond in intermediates **5** and **6** is used as a reference plane to follow the course of the two ring-closure reactions. The two diastereotopic faces of the double bond may be described with *re,si* descriptors[3] for the olefinic carbons. In the case of intermediate **5**, ring closure to the *gem*-dimethylmethano moiety located on the *re-re* side of the reference plane affords the pivalate ester precursor **9** for (3*R*,4*R*)-**1**, while (2*R*,3*R*,4*S*,6*R*)-**7** results from closure to the *si-si* face of the double bond. Compounds **7** and **8** were not expected to be cannabimimetic since at first glance they did not seem to possess the structural determinants of such activity. Preliminary tests, however, showed marked activity in the ring test by both enantiomers (**7** and **8**). Hence we investigated in greater detail their structural features as well as their cannabimimetic activity.

Results

Pharmacology. The results of the tests on compounds **7** and **8** in animals trained to discriminate between the presence and absence of the effects of (−)-Δ^1-THC (**3**) are shown in Figure 1. The response learned in the presence of the THC generalized to the effects induced by compound **8** in both species, the ED_{50} values being 0.72 (0.98) and 0.17 mg/kg (0.99) in rats and pigeons, respectively. The ED_{50} value for compound **7** is 3.57 (0.78) mg/kg in pigeons. The numbers in parentheses indicate the correlation coefficient (*r*) for the logarithmic regression analysis.

Scheme I



† The Hebrew University.
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(1) (a) Mechoulam, R.; Lander, N.; Srebnik, M.; Breuer, A.; Segal, M.; Feigenbaum, J. J.; Järbe, T. U. C.; Hiltunen, A. J.; Consroe, P. In *Structure Activity Relationships of the Cannabinoids*; Rapaka, R. S., Makriyannis, A.; Eds.; NIDA Research Monograph 79, U.S. Department of Health and Human Services: Rockville, MD, 1987, pp 15–30. (b) Mechoulam, R.; Feigenbaum, J. J.; Lander, N.; Segal, M.; Järbe, T. U. C.; Hiltunen, A. J.; Consroe, P. *Experientia (Basel)* **1988**, *44*, 762.

(2) The numbering used for the THC-type compounds is a monoterpene one, as indicated on the formula in Scheme I. In order to facilitate comparisons between the compounds described in this publication, the same numbering system is retained in all formula (including the furans **7** and **8**) although such a numbering does not follow Chemical Abstracts rules.

(3) Hanson, K. R. *J. Am. Chem. Soc.* **1966**, *88*, 2731.

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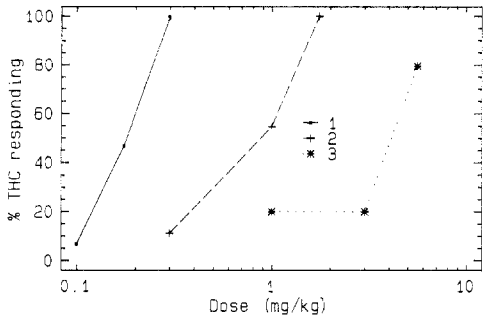
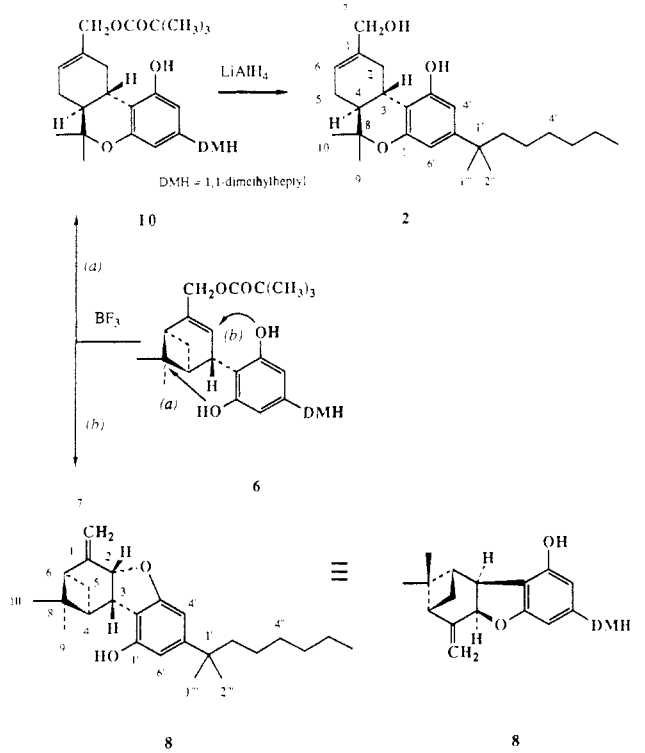


Figure 1. Generalization tests with various doses of the enantiomeric pair **7** and **8** in rats and pigeons trained to discriminate between the presence and absence of the effects induced by (−)-Δ^1-THC. 1 = enantiomer **8** (pigeons, n = 5); 2 = enantiomer **8** (rats, n = 9); 3 = enantiomer **7** (pigeons, n = 5). For ED_{50} and correlation coefficients, see the Results section, in the text; for details of the procedure, see the Experimental Section.

Scheme II



The highest degree of THC-appropriate response for compound **7** in rats was 33% (with 10 mg/kg) and therefore no ED_{50} could be estimated. The ED_{50} values are based on the % RDP [percentage responding to drug (THC) associated position out of the total number of responses emitted in the test] during the test probe yielding the highest % RDP, i.e. the lowest ED_{50} value.

The ED_{50} values for (−)-Δ^1-THC (**3**) are 0.66 mg/kg (0.94) in rats and 0.18 mg/kg in pigeons. These values were determined as described previously (see ref 1b and references cited therein).

The above results show that in the drug-discrimination tests used by us the potency of compound **8** is equivalent to that of Δ^1-THC in both species. Figure 1 furthermore shows that though compound **7** does elicit THC-appropriate responding in the pigeons, the potency difference of the order of 21 between **8** and **7** indicates that a stereochemical differentiation exists within this enantiomeric pair.

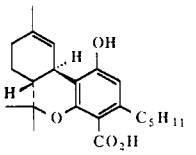
In the mouse ring test (see Table I) enantiomer **8** was found to be considerably more active than Δ^6-THC (**4**); the activity of enantiomer **7**, however, was not significantly different than that of Δ^6-THC.

Table I. Psychotropic Effects of the Enantiomers **7** and **8**, Compared to those of Δ^6-THC (**4**) As Evaluated by the Mouse Ring Test[a]

dose, mg/kg	Δ^6-THC (4)	enantiomer 7	enantiomer 8
vehicle	12.3 ± 2.0 (10)	13.3 ± 2.8 (10)	12.0 ± 3.3 (10)
20		43.2 ± 3.1 (8)*	89.2 ± 3.1 (8)*
10	41.3 ± 5.6 (7)*	28.1 ± 5.0 (6)*	66.1 ± 7.0 (8)*
1	20.9 ± 1.6 (7)*	20.0 ± 3.2 (8)	42.0 ± 4.2 (8)*
0.1	13.2 ± 1.8 (7)	10.0 ± 3.0 (10)	13.2 ± 4.2 (6)

[a] Numbers indicate percentage of time over 5 min a mouse stays immobile on a ring. Numbers in parentheses indicate numbers of animals. Vehicle consists of ethanol–Emulphor-620–water (1:1:18). Differences between drug and vehicle treatments were evaluated by Student's t test. Significant differences ($P < 0.05$) are indicated by asterisks.

Geometrical Models Calculated by Molecular Mechanics. Model compounds for stereochemical comparisons among **1–3**, **7**, and **8** were calculated via the molecular mechanics program MM2 developed by Allinger and coworkers.[4] The X-ray crystallographic coordinates of Δ^1-tetrahydrocannabinolic acid B[5] (**11**) were used as the input structure for the calculation of (−)-Δ^1-THC (**3**).



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In the calculated model structures for **1–3**, an ethyl group was used in place of the n-pentyl substituent, and in those for **7** and **8** a *tert*-butyl group was used in place of the 1,1-dimethylheptyl moiety. With the exception of the abovementioned substituent change at C(5′) and the presence or absence of the carboxylic acid group, the geometry for the MM2-calculated model of Δ^1-THC (**3**) and the X-ray-determined structure for **11** were very similar. Inspection of the two lists of torsion angles for the cyclohexenyl and pyranyl rings (rings A and B, respectively) (as well as those involving the pendant methyl groups) showed that at a 5% level of significance one can conclude that there was no evidence of a difference between the two structures. The energy-minimized model for **3** was used as the starting point for making the structural changes for the models of **1** and **2** prior to final minimization of energy. Moreover, the MM2 models for **1–3** were all consistent with the known NMR parameters for these compounds (vide infra). Our MM2-calculated model for **3** is also very similar to that of Reggio and Mazurek,[6] as well as the earlier structure[7] calculated by the older MM1 method (vide infra). An extensive NMR study of **8** was undertaken to provide an experimental comparison with the MM2 models for **7** and **8**. The experimentally determined NMR $^3J_{H-H}$ coupling constants and nuclear Overhauser effect (NOE) enhancements for **8** were all consistent with the MM2-calculated model compound (vide infra). Comparative views of **11** (X-ray) and the MM2 model structures for **1**, **3**, and **8** are shown in Figures 2 and 3. Selected structural parameters

(4) (a) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127. (b) Allinger, N. L.; Yuh, Y. H. MM2. *QCPE* **1980**, *13*, 395.
(5) Rosenqvist, E.; Ottersen, T. *Acta Chem. Scand.* **1975**, *Ser. B29*, 379.
(6) Reggio, P. H.; Mazurek, A. P. *THEOCHEM* **1987**, *149*, 331.
(7) Archer, R. A.; Boyd, D. B.; Demarco, P. V.; Tyminski, I. J.; Allinger, N. L. *J. Am. Chem. Soc.* **1970**, *92*, 5200.

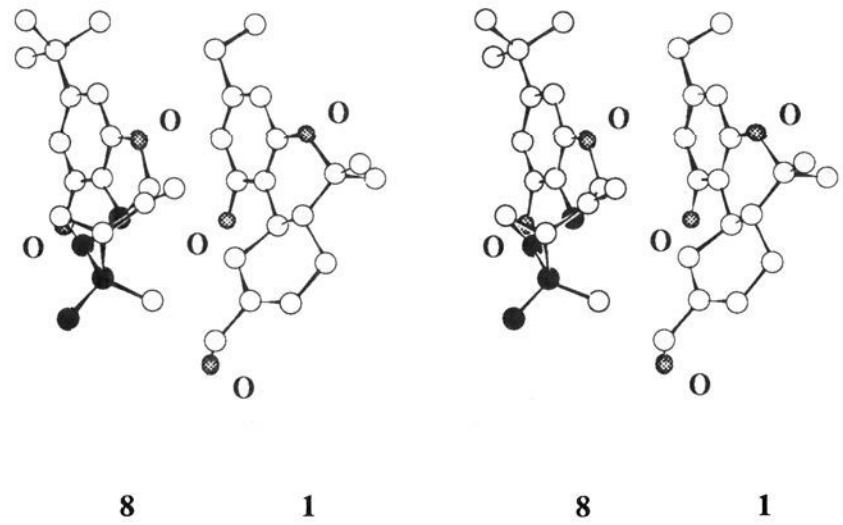

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Figure 2. Stereoview[15] comparison of MM2-calculated models for (3*R*,4*R*)-**1** (on the right), and (2*R*,3*S*,4*S*,6*S*)-**8** (on the left). Ring A is bent into the *si-si* reference face of the resorcinol ring (*si-si* face is oriented toward the left-hand side of each molecule). Atoms C(7), C(1), C(2), and C(3) in the bicyclo[3.1.1]heptane moiety of **8** have been drawn in black for comparison with correspondingly positioned carbons in the cyclohexenyl ring A of **1**. Hydrogen atoms have been omitted for clarity.

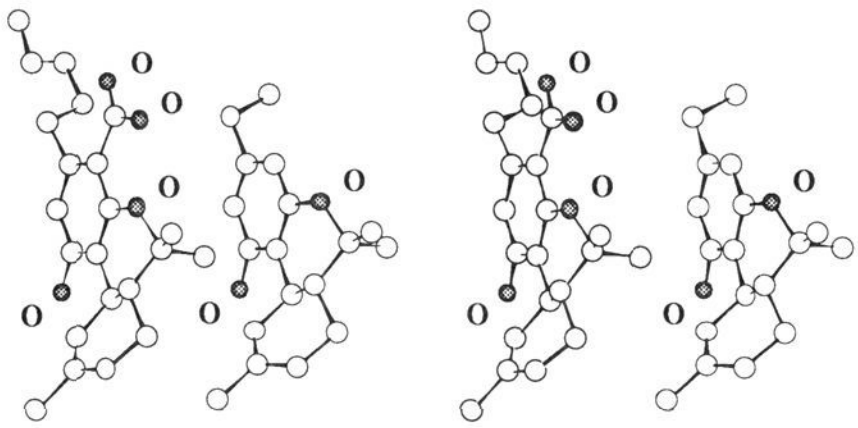

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Figure 3. Stereoview[15] comparison of MM2-calculated model for (3*R*,4*R*)-**3** (on the right) with single-crystal X-ray diffraction structure of (3*R*,4*R*)-**11** (on the left). Ring A is bent into the (*si-si*)-reference face of the resorcinol ring (*si-si* face is oriented toward the left-hand side of each molecule). Hydrogen atoms have been omitted for clarity.

for **1–3**, **7**, and **8** show that part of the bicyclic moiety in **8** is similar to part of the cyclohexenyl ring A in **1** and **3** (vide infra).

^{1}H and ^{13}C NMR Spectral Parameters. The ^{1}H and ^{13}C NMR spectral parameters for **7** and **8** are listed in Table II and in Table III of the supplementary material, respectively. Assignment criteria details are presented to illustrate the complete agreement between MM2-calculated models **7** and **8** and the NMR spectral parameters. Multiplicities of carbon resonances were determined by DEPT (90° and 135° pulse angle) experiments. Assignments of protonated carbons via $^1J_{C-H}$ were made by the HETCOR (XHCORR) 2D NMR correlation technique, and quaternary carbon resonances were assigned via $^3J_{C-H}$ long-range HETCOR correlation to vicinal protons.

The 6.24 ppm aromatic *H* doublet sharpened in a homonuclear decoupling experiment involving the phenolic proton (O*H*) and thus was assigned as H(6′). The lower field chemical shift value for C(6′) versus C(4′) is also in agreement with estimated C(4′,6′) chemical shift values based on phenyl ring substituent effects.[8] The higher field

Table II. ^{1}H NMR Spectral Parameters for **7** and **8**[a]

δ_H, ppm[b]	J_{H-H}, Hz[c]	torsion angle in MM2 model[d]	calcd angle, deg
H(2), 2.33	2–3, 2.2(1)	H(2)–C(2)–C(3)–H(3)	−63
H(3), 3.81	2–6, 5.7(5)	H(2)–C(2)···C(6)–H(6)	−0.5
H(4), 5.23	2–9a, *e*	H(2)–C(2)–C(9)–H(9a)	−99
H(6), 2.60	2–9b, 5.9 (4)	H(2)–C(2)–C(9)–H(9b)	30
C(7)H_3, 1.33	3–4, 8.7 (4)	H(3)–C(3)–C(4)–H(4)	11
C(8)H_3, 0.82	3–6′, *f*		
H(9a), 1.30	3–9b, 1.7 (1)	H(3)–C(3)···C(9)–H(9b)	−54
H(9b), 2.26	4–10a, *g*	H(4)–C(4)···C(10)–C(10a)	41
H(10a), 5.24	6–9a, *e*	H(6)–C(6)–C(9)–H(9a)	97
H(10b), 5.07	6–9b, 5.9 (4)	H(6)–C(6)–C(9)–H(9b)	−31
H(4′), 6.41	6–10a, *f*	H(6)–C(6)···C(10)–H(10a)	−171
H(6′), 6.24	9a–9b, −10.4(2)		
C(2″)H_2, ∼1.50	4′–6′, 1.3 (1)		
C(3″)H_2, ∼1.04	4′–O*H*, *h*		
C(4″–6″)H_2, ∼1.17 ± 0.02	C(6″)H_2–C(7″)H_3, 6.4 (1)		
C(7″)H_3, 0.84			
C(1‴)H_3, 1.20			
C(2‴)H_3, 1.20			

[a] 200 MHz, $CDCl_3$, ambient temperature. [b] Ppm downfield from internal tetramethylsilane. [c] Standard deviation of coupling constants given in parentheses. [d] Torsion angles from molecular mechanics calculated model for **8**. [e] Not observed. [f] Magnitude unable to be determined (observed in both COSY-45 2D-NMR and in 1D-NMR homonuclear decoupling experiments). [g] Magnitude unable to be determined (observed in COSY-45 2D-NMR spectrum). [h] Magnitude unable to be determined (observed in 1D-NMR homonuclear decoupling experiments).

chemical shift [H(10b)] of the two found for the diastereotopic olefinic H(10a,b) pair was assigned as *syn to H(6)* on the basis of a 2.7% nuclear Overhauser effect for the 2.60 ppm signal (H(10b)) and a 3.4% NOE for the 5.07 ppm signal (H(6)). In the model compound, distance H(6)···H(10b) was calculated to be 2.49 Å, in accord with the observed NOE phenomenon.

The vicinal $^3J_{3-4}$ coupling constant of 8.7 (1) Hz is consistent with a synperiplanar arrangement between H(3,4), i.e. cis fusion between the benzofuranyl ring system and the bicyclo[3.1.1]heptane moiety. The H(3) signal shows a small long-range coupling constant with H(5b) ($^4J_{3-9b}$), while as expected, a larger long-range cyclobutane-type $^4J_{2-6}$ coupling constant of 5.7 (5) Hz was noted between the two bridgehead protons. The magnitudes of the vicinal coupling constants in the bicyclo[3.1.1]heptane moiety are consistent with a Karplus-type relationship[9] involving the relevant dihedral angles noted in the molecular mechanics calculated model compound (see Table II).

The lack of discernable vicinal coupling between H(9a) (*syn to the resorcinol ring*) and H(2,6) is consistent with torsion angles of 97° and −99° for H(9a)–C(9)–C(6)–H(6) and H(2)–C(2)–C(9)–H(9a), respectively (in the MM2-calculated model compound). Similarly, the assignment of H(9b) (*anti to the resorcinol ring*) is consistent with the magnitude of 5.9 (5) Hz noted for $^3J_{9b-6}$ and $^3J_{2-9b}$, since the H(9b)–C(9)–C(6)–H(6) and H(2)–C(2)–C(9)–H(9b) torsion angles are −31° and 30° in the model compound. The higher field chemical shift [C(8)H_3] of the two found for the diastereotopic C(7,8)-methyl groups was assigned as *syn to the resorcinol ring* on the basis of difference-NOE experiments: an 8.5% nuclear Overhauser effect enhancement for H(3) and 3.1% for H(4) (0.82 ppm CH_3), while (1.33 ppm CH_3) gave a 6.8% enhancement for H(9b). In the model compound, the minimum distances between

(8) Atta-ur-Rahman *Nuclear Magnetic Resonance, Basic Principles*; Springer-Verlag: New York, 1986; pp 149–160.

(9) (a) Karplus, M. *J. Chem. Phys.* **1959**, *30*, 11. (b) Karplus, M. *J. Am. Chem. Soc.* **1963**, *85*, 2870.

the 0.82 ppm CH_3 and H(3) and H(4) were calculated to be 2.39 and 2.55 Å, respectively, in accord with the observed NOE phenomenon. Irradiation of the accidently equivalent C(1‴,2‴)H_3 afforded NOE enhancements of 10.6% for H(4′) and 9.5% for H(6′).

Homonuclear decoupling of a ca. 1.49 ppm two-proton multiplet showed a change in another two-proton multiplet at ca. 1.04 ppm. Weak NOE (1.49 ppm CH_2) enhancements of ca. 0.4% for both H(4′,6′) were noted, suggesting that the irradiated nuclei were ligated to C(2″) while those at ca. 1.04 ppm are C(3″)H_2. Estimated carbon chemical shift values for side-chain carbons C(2″–7″) based on substituent effects[8] were in good agreement with experimental values for CH_2 resonances, and thus C(2″,3″)H_2 were confirmed via HETCOR.

The singlet phenolic OH signal was assigned on the basis of saturation transfer (4.76 ppm) to a small peak at 1.69 ppm (putative free water). This exchange phenomenon was confirmed by the presence of the appropriate cross peak in the NOESY-45 spectrum.

Discussion

Description of the Benzofuran Structures. In the molecular mechanics calculated model compound, five carbon atoms in the cyclohexane moiety are observed to be approximately coplanar [C(2–5,6)], and thus the conformation of this fragment may be described as a gem-dimethylated half-chair. From the NMR coupling constants and the observation of NOE (C(8)H_3) enhancements of H(3,4), the benzofuran moiety is seen to be cis-fused to the half-chair cyclohexane moiety such that the benzofuranyl rings are located anti to the *gem*-dimethyl-substituted methano bridge. The bent nature of the molecule is seen by the fact that the best plane through carbons C(2,3,5,6) affords a dihedral angle of ca. 62° with the best plane through the benzofuranyl phenyl ring.

Conformational Comparisons between Benzofuran and Benzopyran Cannabinoids. Most compounds which cause cannabimimetic activity are tricyclic, ring C being resorcinol derived and ring B being a pyran. Rings A and C are generally at a low angle to each other. In Δ^1-THC (**3**) itself, the C(1′)–C(2′)–C(3)–C(4) torsion angle was calculated to be 19° by the older MM1 molecular mechanics program[6] and 17° in the X-ray structure of acid **11**.[5] Recent studies by Reggio and Mazurek[6] have further defined the spatial structure of **3**. The few exceptions to the above generalizations are those stemming from compounds which, though considerably different in their chemical structure from the "classical" cannabinoids exemplified by **3** and **4**, can take up closely related conformations.[10]

In most psychotropically active cannabinoids the C(3) carbon atom (located at the junction of rings A and C) is *stereogenic*. The enantiomer in which such a carbon has the same spatial arrangement of ligated groups as found for C(3) in (–)-Δ^1-THC (3*R*) is either the only active enantiomer or significantly more active than the enantiomer with the opposite configuration at this position vis-a-vis **3**.[1] This similarity of ligated group spatial dispositions at the stereogenic atom is distinct from the comparison of Cahn–Ingold–Prelog (CIP)[11] absolute configuration descriptors. For example, both L-serine and L-cysteine have similar C_α absolute configurational arrangements (i.e. they are *homochirally similar*[12,13]), but their CIP descriptors are different (*S* and *R*, respectively).

The enantiomeric pair of compounds (**7** and **8**) discussed in this paper differ from the active cannabinoids (as exemplified by Δ^1-THC) in several respects, the most obvious and significant one being the presence of a benzofuran moiety (rather than a benzopyran one as in THC). Another difference is that the CIP descriptor for C(3) in the more active enantiomer (**8**) is opposite to that in THC, although we will show (vide infra) that the respective configurational arrangements at this atom are actually quite similar. The dimethylheptyl side chain in **7** and **8**, compared to the *n*-pentyl one in **3**, is expected to increase potency.[14] The bicyclic nature of the terpene ring in **7** and **8**, contrasted with the monocyclic terpene moiety in **3**, should not necessarily cause significant changes in activity, as it has previously been established that in many cases major modifications in ring A have not led to elimination of cannabinoid action.[15]

The drug-discrimination test results presented in Figure 1 and those for the mouse ring test in Table I indicate that compound **8** is a potent cannabimimetic; the enantiomeric **7** is considerably less active. One can rationalize these results by comparing the MM2 model structures for **1**, **3**, and **8**. In this section we will show that our MM2-calculated geometries for **1**–**3** models are in agreement with available NMR/X-ray data and with other calculated structures. As was noted above, the MM2 model for Δ^1-THC (**3**) is very similar to the X-ray-determined structure for acid **11** [see Figure 2].[5] The model for **3** also appears to be pictorially very similar to that presented by Reggio and Mazurek[6] using the same MM2 program. Numerical agreement in bonding parameters was also found in those cases where quantitative comparisons between the two models could be made. For example, the C(3)–C(4)–C(8)–O(ether) torsion angle was calculated to be 63° in both models and 61° in **11** (X-ray). The abovementioned 61°–63° C(3)–C(4)–C(8)–O(ether) torsion angles are similar but slightly higher than the 56° value reported by Archer et al.[7] using the older MM1 method. Both Archer et al.[7] and Reggio and Mazurek[6] noted that the cyclohexenyl A ring in **3** should exist predominantly in a half-chair form. Our model structure for **3** is in complete agreement with this as shown by –5° and 3° values for torsion angles C(5)–C(6)–C(1)–C(2) and C(6)–C(1)–C(2)–C(3), respectively [–11° and 3° respectively for **11** (X-ray)]. Our value of 9° for the C(6′)–C(1′)–C(3)–C(4) torsion angle is similar but slightly smaller than the 19° value calculated by the older MM1 method [16° for **11** (X-ray)]. The root mean square (rms) difference between the MM2-calculated set of torsion angles in rings A and B of **3** (plus those involving pendant methyl groups) versus corresponding angles in X-ray-determined **11** is only 4.6°. For the purposes of reference, rms differences of this relatively small magnitude are usually noted for sets of torsion angles in molecules whose geometries are not ideally identical, but yet are very similar, e.g. the molecular structure in a crystalline racemic modification versus that in the chiral crystal.[16]

Our models for both **1** and **3** are also completely consistent with the ^{1}H NMR parameters reported for these

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compounds by Archer et al.[7] The 10.9-Hz vicinal coupling constant reported for H(3)–H(4) in **3** and **4** is in accord with our calculated antiperiplanar H(3)–C(3)–C(4)–H(4) torsion angle [−169° in **1**, −166° in **3**, −178° in **11** (X-ray)]. In addition, the 10.5- and 4.5-Hz coupling constants between H(2-ax)–H(3) and H(2-eq)–H(3), respectively in **4**, agree with the calculated −168° antiperiplanar and −49° synclinal (gauche) values for H(2-ax)–C(2)–C(3)–H(3) and H(2-eq)–C(2)–C(3)–H(3), respectively in **1**. Furthermore, the close proximity of H(3) to the axial methyl protons [axial methyl designated as C(10)] in our models for **1** and **3** is consistent with the NOE enhancement of H(3) (upon irradiation of C(10)H_3) which was observed for both **3** and **4**.[7] This cis-1,3-diaxial relationship results in an *r*(H(3)···C(10)) nonbonding distance of 2.88 and 2.90 Å in our models for **1** and **3**, respectively, compared to 2.89 Å for **3** as reported by Archer et al.[7] [2.76 Å in **11** (X-ray)]. The minimum distance between a C(10)-methyl proton and the H(3) in our models for **1** and **3** is 2.36 and 2.37 Å, respectively, which is clearly commensurate with the NOE observations [2.15 Å in **11** (X-ray)]. The results of our NMR investigations of **7** and **8** are also all consistent with our MM2-calculated model for this compound as has been shown above.

Comparison of Pharmacological Activities and Molecular Structure. On this basis we will proceed to compare the calculated models for the 7-hydroxy-Δ^6-tetrahydrocannabinol enantiomeric pair [(3*R*,4*R*)-**1**/(3*S*,4*S*)-**2**], Δ^1-THC [(3*R*,4*R*)-**3**] and the enantiomeric pair of benzofuran derivatives [(2*R*,3*R*,4*S*,6*R*)-**7**/(2*S*,3*S*,4*R*,6*S*)-**8**]. Ring C has diastereotopic faces to which *re,si* descriptors[3] may be assigned. For the assignment of these descriptors, the adjacent trigonal carbon atoms C(1′) and C(2′) were chosen to define the reference plane for **1–3**, **7**, and **8**. Thus, a *si-si* descriptor was determined for the ring-C faces of **1**, **3**, **8**, and **11** tilting toward the viewer in Figures 2 and 3. This aromatic (*si-si*) face will be used as a reference face for **11** [X-ray] and for MM2 models of **1–3**, **7**, **and 8**. A perpendicular was calculated to the midpoint (m) of ring C, and a point (m′) was then located on the portion of this line segment passing through the *si-si* face of the reference plane. Nonbonding angles θ for atoms (X) in ring A were calculated in respect to the m′–m line segment in the *si-si* face [i.e. $\theta = \angle$(m′–m···X)], and signed angles (θ') were obtained from $\theta' = 90 - \theta$. Thus, the angular deviation from the aromatic ring of X-atoms located syn to the *si-si* aromatic face will afford positive values of θ', while X-atoms located anti to that reference face will show negative values for this angle. Similarly, an indication of the disposition of X atoms in regard to the *si-si* reference face and their position relative to a plane perpendicular to ring C and bisecting the two C-substituted aromatic ring atoms is given by torsion angle (ϕ) = C(6′)–C(1′)–m···X for **1–3**, **7**, and **8**. As before, negative ϕ values point to a syn disposition of the X atom in regard to the *si-si* aromatic face, and values approaching ±180° indicate a more distant relationship of the X atom relative to the aromatic ring bisection plane.

The C(3)–C(2)–C(1)–C(7) chain of carbon atoms attached to the resorcinol ring in the series **1–3**, **7**, and **8** is reasonably equivalent in terms of the respective distances of corresponding atoms to the midpoint of the aromatic ring [methyl(ol) groups terminate these chains]. The mean values for the nonbonding *r*(m···X) distances in the models for **1**, **3**, and **8** are (Å, standard deviation in parentheses) 2.92 (2), X = C(3); 3.82 (3), X = C(2); 5.19 (7), X = C(1); and 6.33 (7), X = C(7). In addition, this chain of carbon atoms in models for **1**, **3**, and **8** show equivalent *signs* and magnitudes for their respective angles of deviation (θ') relative to the *si-si* face of that plane. The mean values for angle θ' in **1**, **3**, and **8** (with standard deviation in parenthesis) are +0.5 (7)°, X = C(3); +18 (3)°, X = C(2); +15.7 (8)°, X = C(1); and +22 (2)°, X = C(7). Corresponding carbon atoms in the respective enantiomers (**2** and **7**) show equivalent magnitudes but *opposite signs* for the respective angles of deviation. Thus, for **1** and **8** the carbon atoms in the abovementioned chain all reside on the same side as the *si-si* reference face of the aromatic ring. This is also found for (−)-Δ^1-THC (**3**), while in their enantiomeric counterparts (**2** and **7**) this chain of carbon atoms is oriented toward the opposite *re-re* side. Similarly, the disposition of H(3) in both **1** and in **8** fall in the same *re-re* plane as does H(3) in (−)-Δ^1-THC (**3**). Thus, the spatial arrangement of atoms in the vicinity of the free phenolic group of **1** and **8** shows similarities to that for (−)-Δ^1-THC (**3**). It is generally recognized that the free phenolic group and its vicinity are of importance in cannabinoid activity.[14,15]

Torsion angles (ϕ) for the above-mentioned carbon atom chain in ring A show *negative* values for **1**, **3**, and **8** while *oppositely signed* values are noted for the corresponding enantiomers **2** and **7**. The ϕ values in **1**, **3**, and **8**, respectively, are −83°, −23°, +2° for X = C(3); −125°, −132°, −95° for X = C(2); −117°, −115°, −72° for X = C(1); and −129°, −123°, −95° for X = C(7). This also shows that rings A in **1** and **8** are bent toward the same *si-si* face of the resorcinol ring as that in Δ^1-THC (**3**), while the corresponding rings A in the enantiomeric compounds **2** and **7** are disposed toward the opposite side of the molecule. The smaller magnitudes for torsion angles (ϕ) for the C(3)–C(2)–C(1)–C(7) edge of ring A in **7** and **8** relative to corresponding values in **1–3** illustrate that ring A is located closer to the ring-C bisection plane when a furanyl ring replaces the pyranyl moiety in Δ^1-THC (**3**).

During revision of this paper, a paper by Reggio et al.[17] appeared in the literature discussing the importance of the substituent C(7) orientation to cannabinoid activity [C(7) is the numbering system in our paper]. Using a series of (3*R*)-benzopyran-type cannabinoid structural models calculated by the MM2P(85)[18] program, they correlated negative values for the C(7)–C(1)···C(3′)–*O*H torsion angle with cannabinoid activity, e.g. −49° for (−)-Δ^1-THC (**3**) [−50° in our model **3**].[17,19] This was discussed in terms of C(7) protruding into the "top face" of the molecule. Their "top face of the molecule"[17] is the same as our *si-si* descriptor for the aromatic reference plane. The "protrusion angle"[17] in models for our active benzopyran **1** and active benzofuran **8** is −41° and −46°, respectively. Reggio et al.[17] also compared the C(7)–C(1)···C(3′)–*O*H torsion angles in models for epimeric axial-methyl versus equatorial-methyl saturated ring A analgoues of **3**. They noted that the active equatorial-C(7) compound showed a −54° value, while that for the low activity axial-C(7) epimer was +48°.[17] The lowered activity of the axial-methyl epimer was ascribed to methyl group protrusion into the "bottom face" of the molecule (our *re-re* face),

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(19) The geometry of our MMP2 calculated model for **3** is the same as that determined by "MM2P(85)" in ref 17. Five of the ring A torsion angles are each only one degree lower in magnitude but agree in sign, and the sixth, [the synperiplanar C(3)–C(2)–C(1)–C(6) angle] is +1° versus +4° in ref 17.

which "may hamper or prevent molecules from binding at their site of action".[17] We note that while methyl C(7) in benzofuran 8 protrudes into the *si-si* face and methyl C(8) protrudes into the *re-re* face under the aromatic ring, this compound is active [+78° C(8)–C(1)···C(3′)–*O*H torsion angle].

In summary, compounds 1 and 8 contain similar structural features to those found in Δ^1-THC (3), while those for the enantiomeric counterparts 2 and 7 differ due to external enantiotopicity. The finding of similar stereochemical features in 1 and 8 relative to those in Δ^1-THC (3) may now be correlated with the observations of higher cannabimimetic activities for these molecules relative to those noted for their respective enantiomers (2 and 7). Although the structures of 7 and 8 differ considerably from those of the active cannabinoids, the observation that 8 is much more potent than 7 in two different types of pharmacological tests can be reasonably explained on the basis of the general stereochemical requirements for activity within the cannabinoid class of compounds.

Experimental Section

Chemistry. Melting points were taken in glass capillary tubes with a Thomas-Hoover Uni-Melt apparatus. Infrared spectra were recorded on a JASCO A-200 spectrophotometer. Rotations were determined on a Perkin-Elmer Model 141 polarimeter in chloroform. The microanalyses were performed by the Microanalytical Laboratory of the Hebrew University and the elemental compositions of the compounds agreed to with ±0.4% of the calculated value. Chromatographic separations were performed on silica gel columns (Woelm TSC silica, for dry chromatography, activity III/30 mm, No. 04530).

Synthesis of Compounds 7 and 8. (2*R*,3*R*,4*S*,6*R*)-Benzofuran 7. (2*R*,3*R*,6*R*)-Compound 5[1] (2.7 g) was dissolved in dry methylene chloride (100 mL, freshly distilled over calcium hydride). Boron trifluoride etherate (2.7 mL) was added and the solution was stirred for 30 min under nitrogen. The solution was diluted with ether and washed with aqueous sodium bicarbonate. The organic phase was washed with water and dried over anhydrous magnesium sulfate, and the solvent was evaporated under reduced pressure. The oil obtained (2.5 g) was chromatographed on silica gel. Elution with 5% ether in petroleum ether gave compound (2*R*,3*R*,4*S*,6*R*)-7 (700 mg) which was recrystallized from pentane to give crystals: mp 156–158 °C; $[\alpha]_D$ +178° ($CHCl_3$). The ^1H and ^{13}C NMR spectral parameters are given in Table II and Table III (supplementary material), respectively. Anal. ($C_{25}H_{36}O_2$) C, H.

(2*S*,3*S*,4*R*,6*S*)-Benzofuran 8. The enantiomeric 8 is prepared exactly as described above for 7 except that (2*S*,3*S*,6*S*)-compound 6[1] is used as starting material. All the physical constants and spectra of (2*S*,3*S*,4*R*,6*S*)-8 are identical with those of 7 except for the rotation, which is $[\alpha]_D$ −175° ($CHCl_3$).

Nuclear Magnetic Resonance Spectroscopy and Molecular Mechanics Calculations. ^1H NMR spectra ($CDCl_3$) were recorded at 200.1 and 300.1 MHz on Bruker WP-200-SY and AM-300 Fourier transform spectrometers (4.7 and 7.0 T, respectively). ^{13}C NMR spectra ($CDCl_3$) were recorded at 50.3 MHz on the Bruker WP-200-SY instrument with broad band proton decoupling. The deuterated solvent was used as an internal lock, and tetramethylsilane was used as an internal reference for ^1H NMR spectra. Residual $CHCl_3$ protio solvent absorbances were used as internal secondary references (relative to tetramethylsilane) for ^{13}C NMR spectra. The homonuclear spin-spin proton coupling networks were ascertained via a COSY-45 2D-NMR correlation spectrum. Carbon multiplicities were determined by the DEPT pulse sequence using either 90° or 135° pulse angles. Heterocorrelation 2D-NMR spectra utilizing $^1J_{C-H}$ coupling were performed with Δ_1 and Δ_2 delays of 0.0037 and 0.0022 s, respectively, and the corresponding values for long-range $^nJ_{C-H}$ coupling were 0.045 and 0.030 s. Nuclear Overhauser effects were searched for via a NOESY-45 2D-NMR experiment and confirmed in difference-NOE experiments.

The minimized-energy geometry of the molecular mechanics calculated model compounds were determined by the MM2[6] program and were performed on a Micro VAX-II computer under MicroVMS V4.5. The structures in Figures 2 and 3 were drawn with the BALL AND STICK 2.0 program.[20]

Drug Discrimination Training. Seven male White Carneaux pigeons (Palmetto, Sumter, U.S.A.) and 16 male Sprague–Dawley rats (ALAB AB, Sollentuna, Sweden) were trained to discriminate between (−)-Δ^1-THC and vehicle [5% propylene glycol, 2% Tween-80, and 93% physiologic saline (v/v)] according to published procedures.[21,22] The training dose was 0.56 mg/kg (1 mL/kg) administered intramuscularly (im) 90 min prior to the onset of the training session in the pigeons. The training dose of (−)-Δ^1-THC in the rats was 3 mg/kg (2 mL/kg) administered intraperitoneally (ip) 30 min prior to session onset. Which key (pigeons) or lever (rats) was correct on a given training session depended on whether (−)-Δ^1-THC or vehicle had been administered prior to the session. The schedule of reinforcement used was fixed ratio 15 (FR 15) for pigeons and FR 10 for rats. The program ended when the pigeons had received 52 rewards or 20 min had elapsed, whichever occurred first. For the rats the program ended after 15 min irrespective of the number of reinforcements earned. The pigeons were trained 3 days a week and the rats 5 days a week.

Drug-Discrimination Testing. Once trained five of the pigeons were tested once a week (Fridays) with compounds 7 and 8 provided that correct base-line responding was maintained during the regular training sessions occurring on Mondays and Wednesdays. For the rats (n = 9 or 10) there were three tests each 2-week period. The test program ended after six rewards had been obtained or 15 (rats) or 20 min (pigeons) had elapsed since the initiation of the test. Both keys/levers were operable throughout the test probe of six trials and consequently pecking/pressing on either of the two keys/levers produced the 4-s access to the reward. Several intervals, viz. 30, 90, 270, and 540 min in pigeons, and 30, 90, 270, and 390 min in rats, after a single administration were examined after each drug dose. During the period between the test intervals the animals were in their home boxes. Test drugs and dosages were also given im in pigeons and ip in rats and were studied in an unsystematic mixed order. Suspensions were prepared shortly prior to administration and volumes varied, depending upon the amounts to be examined.

Enantiomer 8 was tested at three dose levels in each species: 0.3, 1.0, and 1.75 mg/kg in the rat and 0.1, 0.175, and 0.30 mg/kg in the pigeon. Enantiomer 7 was tested with 3.0, 5.6, and 10 mg/kg in the rat and 1.0, 3.0, and 5.6 mg/kg in the pigeon. For the experimental details of the testing of (−)-Δ^1-THC, see refs 1b, 18, and 19.

Mouse Ring Test. For the mouse ring immobility assay[23] Sabra strain male mice (20–30 g) (Hebrew University Animal House, Jerusalem) were used. The cannabinoid was dissolved in ethanol and Emulphor 620 and then doubly distilled water was added. The ratio of the solvents (v/v) in the final solution was 5:5:90; injections were ip. The assay measures the percentage of time spent immobile on a horizontal ring of 5.5-cm diameter during a 5-min exposure.

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Supplementary Material Available: ^{13}C NMR spectral parameters for 7 and 8 (Table III) and selected nonbonding distances between ring-A atoms and the midpoint of ring C, nonbonding angular deviation from the plane of ring C, a torsion angle deviation from the plane of ring C, and the corresponding values for 11 (X-ray) (Table IV) (3 pages). Ordering information is given on any current masthead page.

Binding of *cis*-(1,2-Diaminocyclohexane)platinum(II) and Its Derivatives to Duplex DNA

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A theoretical study is presented for the binding of *RR*, *SS*, *SR*, and *RS* isomers 1,2-diaminocyclohexane (DAC) or *cis*-Pt^{II}(DAC) to DNA. *cis*-Pt^{II}(DAC) is ligated to N7(G) on two adjacent intrastrand guanine bases in a kinked pentamer duplex of DNA (AT, CG*, CG*, GC, AT). The relative stability of the complexes is determined by calculating the relative conformational energy of the *cis*-Pt^{II}(DAC)(DNA) complexes with molecular mechanics (MM) and the intrinsic binding or ligation energy with quantum mechanics (QM). The results suggest that the *RR* and *SS* isomers of Pt^{II}(DAC) adducts with DNA are more stable than the *SR*/*RS* isomer by 1.7 kcal/mol relative to the *cis*-Pt^{II}-(DAC$(H_2O)_2$ aquated species. Calculations on the overall stability of these isomers show that the *SS* and *RR* isomers are 6.5–8.2 kcal/mol more stable than the *SR*/*RS* isomers when bound to DNA, and this is attributed to differences in the strain energy in the DAC rings. The theoretical analyses of these compounds correlate a small differential activity with the trend in intrinsic binding energies. The *RR* isomer is more active in B16 melanoma cells, and the *SS* is most active in L1210 leukemia, and in general the *RR* and *SS* isomers are more active than the *SR* and *RS* in most cell types. The fact that the activity is DNA dependent suggests that excision or repair mechanisms may be taking place and that additional mechanistic steps beyond molecular modeling and quantum mechanical calculations are required to fully understand the activity. These studies of molecular fit of *cis*-Pt^{II}(DAC) to DNA are used to suggest substituted DAC compounds that may yield similar binding characteristics. Modifications to yield DAC derivatives are recommended in anticipation that they may also exhibit activity.

Since the discovery of the activity of *cis*-diamminedichloroplatinum(II) in 1969 by Rosenberg,[1] a search has been made for other compounds that will exhibit antitumor activity. For the parent *cis*-$Pt^{II}(NH_3)_2$ compound, the active site is bidentate addition of platinum to N7 atoms of adjacent guanine moieties within the DNA molecule.[2,3] This knowledge was utilized in preparation of the isomeric series of 1,2-diaminocyclohexane (DAC) compounds. The DAC compounds exhibit lower toxicity and a lack of cross resistance over the parent compound while maintaining good activity.[4-6] These compounds exist in more than one isomeric form: as the *SS*, *RR*, and the equivalent *SR*/*RS* forms of the cyclohexane ring in its attachment through the amines to Pt(II), as shown in Figure 1. In different tumor cell types, these isomeric compounds exhibit varying levels of antitumor activity. The entire series exhibits antitumor activity, but the *RR* and *SS* forms are, in general, more active than the *SR* and *RS* isomers. This variance in activity is demonstrated by the following: the *RR* isomer is most active in B16 melanoma cells, while the *SS* isomer shows the greater activity in L1210 leukemia.[7,8] In an attempt to understand the difference in activity shown for these isomers, the *cis*-Pt^{II}(DAC) and *cis*-Pt^{II}(DAC)-(DNA) adducts are studied with molecular mechanics (MM) to examine the conformational effects, and with quantum mechanics (QM) to analyze the ligand binding energy of these complexes.

The relative binding energies of these compounds to DNA were investigated as one possible factor in the activity of these compounds and the molecular fit into the DNA as a second. We previously introduced a pentamer duplex model to account for the molecular fit of a series of substituted Pt(II) diammines.[9,10] This intermediate conformation or IC pentamer duplex (AT, CG*, CG*, GC, AT) allowed for an approximately square-planar complex. The success of the IC (intermediate conformation) kinked

Table I. Relative Molecular Mechanics Energies of Pt^{II}(DAC) Bound to Conformation IC in Duplex DNA[a]

data	*RR*	*SS*	*SR*	*RS*
TE	−2006	−2006	−2006	−2006
U	−489	−497	−495	−487
Q	−1945	−1943	−1941	−1941
T	172	173	175	173
R	24	24	23	23
A	232	237	231	226

[a] The total energy (TE) is the sum of steric (*U*), Coulombic (*Q*), torsional (*T*), stretching (*R*), and bending (*A*) contributions.

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