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Conformational energy downward driver (CEDD): Characterization and calibration of the method

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Summary

A method has been developed that allows one to drive a molecule to conformations of lowest energy given the starting conformation, the identity of the rotatable bonds and the step size. This method has proved useful in our hands in the drug design arena where it is frequently more important to get 'low-energy' conformers of a molecule that match some other (e.g. pharmacophoric or enzyme pocket) requirements than to exhaustively enumerate all possible low-energy conformations for each of the molecules to be studied. The method has been shown to work in the test cases studied to date. Furthermore, so far it has been shown to be sufficiently fast to be used for molecules containing up to 70 rotatable bonds.

Introduction

Conformational analysis has long been recognized as an important problem in physical and computational chemistry [1]. The early recognition that flexible molecules are composed of populations of low-energy conformations has influenced the way chemists think about such diverse subjects as NMR, chemical synthesis and drug design [2]. The emphasis in all of the commonly used conformational analysis techniques is on how to get the 'complete' set of all low-energy conformations within a given energy window for the flexible molecule that is being analyzed. The usual approach to computing these conformations is to modify the torsional angles of interest in some way and then to recompute the energy of the resulting new conformation. This effort is normally quite a laborious one and as a result is sometimes overlooked entirely. Needless to say, the results and conclusions are sometimes seriously compromised as a direct consequence of this oversight.

Occasionally, it is sufficient for the study at hand to obtain an answer to an inherently simpler question. This question is: 'Given a *particular* conformation in a particular environment, can one manipulate torsional angles to arrive at another conformation that is lower in energy than the starting one?'. A related question, if one can succeed, is: 'How much lower in energy is this new conformation?'.

Examples of situations where such an approach could be useful can be found in the area of drug design. One may have a pharmacophoric model for activity in a given assay and, using that model, one may be able to obtain a conformation of a new flexible molecule that matches this pharmacophore. If one were to subject this conformation to such a method and arrive at another conformation that no longer matched the pharmacophore but that was considerably lower in energy, then one could conclude that the molecule would not prefer to take up the proposed conformation and therefore would probably not display the desired activity, assuming of course that the conformations were all interconvertible under physiological conditions. Several possible conformations which all satisfy the pharmacophoric hypothesis could be set up and examined in this way. On the other hand, if one were unable to drive the energy to lower values by such a method then one would have more confidence in predicting that the molecule had a good likelihood of displaying the activity. Of course, these conclusions are predicated on the assumption that the pharmacophoric hypothesis is indeed a reliable one. Such a method may be referred to as a 'conformational energy downward driver' (CEDD). It is important to point out that this is a different tech-

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TABLE 1
CONFORMATIONAL ENERGIES OF 74 TEST MOLECULES

Molecule	Crystal h.o.f[a]	Crystal energy[b]	MinCry h.o.f.[c]	MinCry energy	Run1 h.o.f.	Energy run1	run2	run3	run4	Elapsed time[d]	Rot. bonds
AAXTHP	−321.48	79.47	−315.37	32.39	−315.95	32.35	31.36	32.70	32.36	179	10
ABAXES	−141.36	80.15	−130.77	33.66	−130.83	33.61	32.57	33.62	32.62	35	2
ABBUMO10	−21.28	39.14	−27.83	15.07	−27.69	15.04	15.03	15.04	15.03	17	1
ABINOR02	−235.39	5.22	−235.95	1.74	−236.01	1.71	1.84	1.71	1.49	27	4
ABINOS01	−238.04	5.10	−234.61	1.77	−234.58	1.75	1.83	1.57	1.59	27	4
ABTOET	−174.15	34.25	−194.97	10.12	−194.01	9.26	9.17	9.57	9.65	137	6
ABZTCX	14.76	242.05	8.48	42.72	8.80	42.31	42.78	42.47	42.74	11	
ACADOS	−86.25	74.09	−78.84	51.09	−79.18	51.03	49.84	51.22	49.10	118	7
ACAFLR	55.58	35.82	26.01	14.98	25.50	14.93	14.87	14.93	16.79	19	2
ACANIL01	8.62	21.24	−9.85	5.93	−9.75	5.89	5.75	5.89	5.75	12	2
ACARAP	−367.85	30.13	−367.47	10.69	−367.40	10.66	10.54	10.66	62.33	81	7
ACBNZA01	−20.62	22.13	−40.58	8.69	−40.50	8.65	7.75	8.66	7.76	27	4
ACBUOL	−118.38	49.56	−131.65	11.99	−132.69	10.97	10.99	10.59	9.22	245	12
ACCITR10	−114.97	56.95	−111.25	22.02	−113.72	18.96	21.99	22.28	22.27	26	2
ACDXUR	−203.71	61.02	−169.27	22.73	−168.78	22.68	22.91	22.69	22.93	50	5
ACFPCH	−45.83	25.65	−64.45	6.05	−64.51	6.01	5.98	6.15	9.71	31	3
ACFUCN	−134.83	18.06	−144.25	12.15	−144.28	11.35	11.06	11.40	11.06	81	5
ACGLSP	−399.40	52.59	−402.87	21.21	−402.53	21.16	20.78	21.16	21.42	213	9
ACGLUA11	−270.96	11.60	−267.88	4.18	−269.51	3.89	3.12	3.90	3.10	94	7
ACHGAL	−280.39	27.38	−277.65	17.08	−277.39	17.05	16.29	16.67	16.27	69	5
ACHIST20	−53.92	38.43	−55.60	23.23	−53.32	22.54	22.91	22.88	23.50	68	6
ACHNAP10	−63.28	16.39	−62.88	7.26	−63.10	7.23	7.23	7.23	7.23	9	2
ACHTAR10	−38.75	19.43	−39.74	9.63	−39.93	9.58	9.26	9.38	9.22	30	4
ACIMDC	32.63	8.41	28.08	3.27	27.60	1.51	1.91	1.44	1.43	9	1
ACINDN	−37.52	48.01	−40.44	17.33	−40.95	17.30	17.29	16.78	16.77	27	1
ACINST	−383.58	35.67	−382.69	14.72	−381.69	11.20	10.90	11.08	10.56	120	10
ACKYNU	−111.56	27.92	−129.57	6.06	−129.43	6.02	5.35	5.78	6.51	74	8
ACMBPN	−236.18	32.28	−227.82	12.04	−232.63	11.50	11.23	11.53	11.47	150	8
ACMEBZ	−123.93	19.27	−137.56	5.15	−136.48	4.78	5.74	4.78	4.87	78	4
ACMTDE	−82.70	23.13	−77.75	7.98	−74.00	6.72	5.57	7.10	8.66	180	9
ACNORT	−152.67	274.37	−156.82	35.26	−158.04	33.16	35.34	35.21	35.34	75	5
ACNPAC10	24.19	51.45	14.99	17.93	14.91	17.91	17.89	17.91	17.89	15	2
ACNPEC	−20.03	95.05	−58.99	12.18	−64.70	8.87	5.27	9.55	6.12	135	10
ACONTN10	−214.56	115.94	−208.87	62.45	−207.89	56.86	56.89	57.21	57.19	151	7
ACPENC10	−51.42	100.33	−24.02	47.27	−23.62	41.27	41.69	41.86	41.62	31	4
ACPPCA	−124.90	16.43	−126.41	7.20	−126.39	7.15	6.98	7.15	6.98	24	3
ACPRET03	−230.33	43.87	−214.54	22.70	−214.95	22.67	21.79	22.67	22.30	129	5
ACPYNS	−272.03	30.15	−275.54	17.31	−275.72	17.27	17.64	17.27	17.64	71	5
ACRAMS	91.52	182.94	223.37	38.89	222.72	36.92	22.50	38.82	25.30	104	4
ACSALA01	−113.13	18.84	−132.23	4.54	−132.09	4.48	5.44	4.52	5.85	13	4
ACSESO10	−35.39	141.50	−94.62	50.75	−94.87	50.69	50.29	50.69	50.42	31	2
ACTAND	−113.70	42.40	−98.53	21.72	−98.60	20.49	20.54	20.49	20.49	34	2
ACTHBZ	17.85	37.41	−10.34	15.88	−9.99	15.82	16.43	15.85	17.60	92	6
ACTOLD	2.37	18.77	−16.76	6.40	−16.83	6.36	7.32	6.36	7.32	14	2
ACTYSN	−129.45	19.23	−143.55	5.48	−140.53	4.84	3.96	5.26	4.01	89	7
ACURID	−205.19	54.24	−166.50	19.15	−161.00	18.53	18.59	19.03	18.66	74	5
ACVCHO	−87.50	7.37	−84.75	2.98	−84.84	2.94	3.66	2.94	3.78	34	3
ACXMOL	−334.67	27.73	−332.38	11.41	−331.88	10.40	10.55	10.40	10.55	56	5
ACXMPR	−186.20	18.86	−188.48	10.62	−192.01	8.94	9.12	9.00	9.81	46	6
ACYGLY11	−128.02	5.70	−130.50	3.55	−128.04	3.28	3.24	3.53	3.51	37	4
ACYTID	−148.16	33.71	−112.09	17.75	−112.60	14.72	14.74	14.89	14.85	85	6
ADELOX10	−126.78	63.72	−126.94	25.14	−127.31	25.09	24.96	25.09	24.96	87	5
ADENOS10	−52.50	85.17	−42.88	47.48	−35.45	46.80	46.66	47.16	47.41	91	6
ADFGLP	−210.47	19.36	−211.97	11.44	−212.16	11.42	11.46	11.42	11.48	8	1
ADGSMH	−445.96	49.83	−445.39	28.77	−445.60	28.74	39.16	28.75	29.60	218	10
ADHELA10	−175.74	35.73	−152.06	12.54	−152.77	12.31	12.48	12.52	16.91	38	6
ADMANN	−287.60	11.89	−281.35	3.09	−284.89	1.92	1.92	1.92	1.82	68	6
ADMHEP	−331.68	9.45	−327.62	2.44	−329.40	1.69	1.65	1.52	1.37	102	8
ADMINA	97.46	58.61	87.54	30.10	87.12	30.07	30.09	30.07	30.09	9	1
ADMOPM	−105.80	91.80	−46.55	39.05	−41.09	35.55	36.47	35.51	36.51	159	8
ADRTAR	−87.07	20.47	−98.72	6.06	−103.90	3.63	3.51	4.07	3.69	79	6

TABLE 1
(continued)

Molecule	Crystal h.o.f.[a]	Crystal energy[b]	MinCry h.o.f.[c]	MinCry energy	Run1 h.o.f.	Energy run1	run2	run3	run4	Elapsed time[d]	Rot. bonds
ADYPNL	202.44	80.35	133.77	16.17	133.60	16.11	14.56	16.12	14.59	125	5
AEBDOD10	−7.27	36.52	−10.11	16.27	−9.41	16.24	16.30	16.24	16.30	14	1
AENLAN10	−48.25	134.29	−42.01	83.59	−42.02	83.55	81.18	83.55	81.19	164	7
AFCYDP	−293.08	42.33	−330.98	25.45	553.05	6.81	13.41	12.29	13.47	159	8
AFMSCY	−108.78	58.83	−53.08	25.41	−52.21	25.37	25.42	25.63	25.62	55	5
AFURPO10	−5.73	20.38	−5.40	20.35	186.47	12.35	12.48	12.62	12.48	8	1
AFUTDZ10	71.40	16.63	207.53	9.67	208.64	9.62	9.63	9.63	9.64	4	1
AFUTHU	−90.75	37.29	−52.36	14.31	−53.26	13.92	13.99	13.64	13.99	27	3
AGALAM10	−269.94	9.23	−267.77	3.36	−267.71	3.33	3.06	3.45	2.99	94	7
AGLUAM10	−174.34	9.64	−175.64	4.52	−175.85	4.11	3.85	3.80	3.90	87	8
AHARFU	−116.38	48.48	−69.66	19.96	−69.81	19.93	19.93	20.30	19.79	40	3
AHCDLA	−91.25	17.06	−86.94	9.74	−87.16	9.72	9.76	9.72	9.73	6	1
AHDITX	−124.51	65.37	−92.95	26.88	−92.62	26.85	26.51	26.43	26.61	35	2

All energies and heats of formation are given in kcal/mol.
[a] AM1 heat of formation of the crystal conformation.
[b] TRIPOS force field energy of the crystal conformation.
[c] AM1 heat of formation of the crystal conformation after minimization in the TRIPOS force field.
[d] Elapsed time is the number of wallclock seconds for run1.

nique than simply minimizing the energy of a molecule. The simple minimization of a molecule will only drive a torsion to another value if there is no energy barrier between the current torsional conformation and the new one. A conformational energy downward driver would not be bound by that restriction. It samples conformations even over a torsional barrier. It would, however, be expected to yield results that could depend on the starting conformation. In this sense the method (like all other conformational analysis methods) cannot be viewed as a method for finding the global minimum.

Such a downward driver has been alluded to in the literature [3] without extensive evaluation, and it appeared to be of great value to us. It was therefore implemented into SYBYL [4] via the SYBYL Programming Language (SPL) interface. This paper describes this implementation and the calibration of the program as we have implemented it against a standard set of small molecules [5].

Methods

The general strategy for a CEDD, described previously [3], is to take the rotatable bonds of a flexible molecule one at a time and increment their value by a predetermined step size. At each position the energy of the whole molecule in this new torsional state is computed. The first rotatable bond is examined in this way through an entire 360° rotation. Then it is fixed at the value that produced the lowest energy. The next designated rotatable bond is then examined with the first one fixed at its 'minimum' energy value. A minimum energy value is determined for the second and it is in turn fixed to that value, and so on, until all of the bonds have been examined and fixed. At this point, the method is said to have completed one iteration. Iterations continue in this way until none of the torsions are changed for a pair of consecutive iterations.

Some traditional methods of exhaustive conformational searching become very expensive in terms of computer time as the number of rotatable bonds increases. For example, in a Combinatorial Search approach, *if one fixes the step size* that one will use for a given search, then the number of conformations that the method will generate and evaluate is given by the formula

$$N = \sum (360/s)^n$$

where N is the number of conformations evaluated, s is the step size and n is the number of rotatable bonds.

The method described here does not have this type of strong dependance on the number of rotatable bonds in the system. In fact, it has proved quite practical to run all of the molecules reported here (and also several molecules with up to 25 rotatable bonds) interactively. By this term we mean that a user has no need to submit such a computation to a batch stream. Since this is a subjective term that means different amounts of elapsed time to different people, a column showing the elapsed time for each of the molecules has been included in Table 1.

The dependance of the result on the step size was a concern to us. Clearly, if one takes very large step sizes then the possibility exists that the method will completely miss nearby minima. If the step size is too small, the method can take too long to be practical as set up. We have tried several different step sizes and apart from these obvious findings have not observed much dependance of the outcome on the step size. The intent here is to simply describe a method that works for the cases studied and that we find useful in our work and to offer it to the

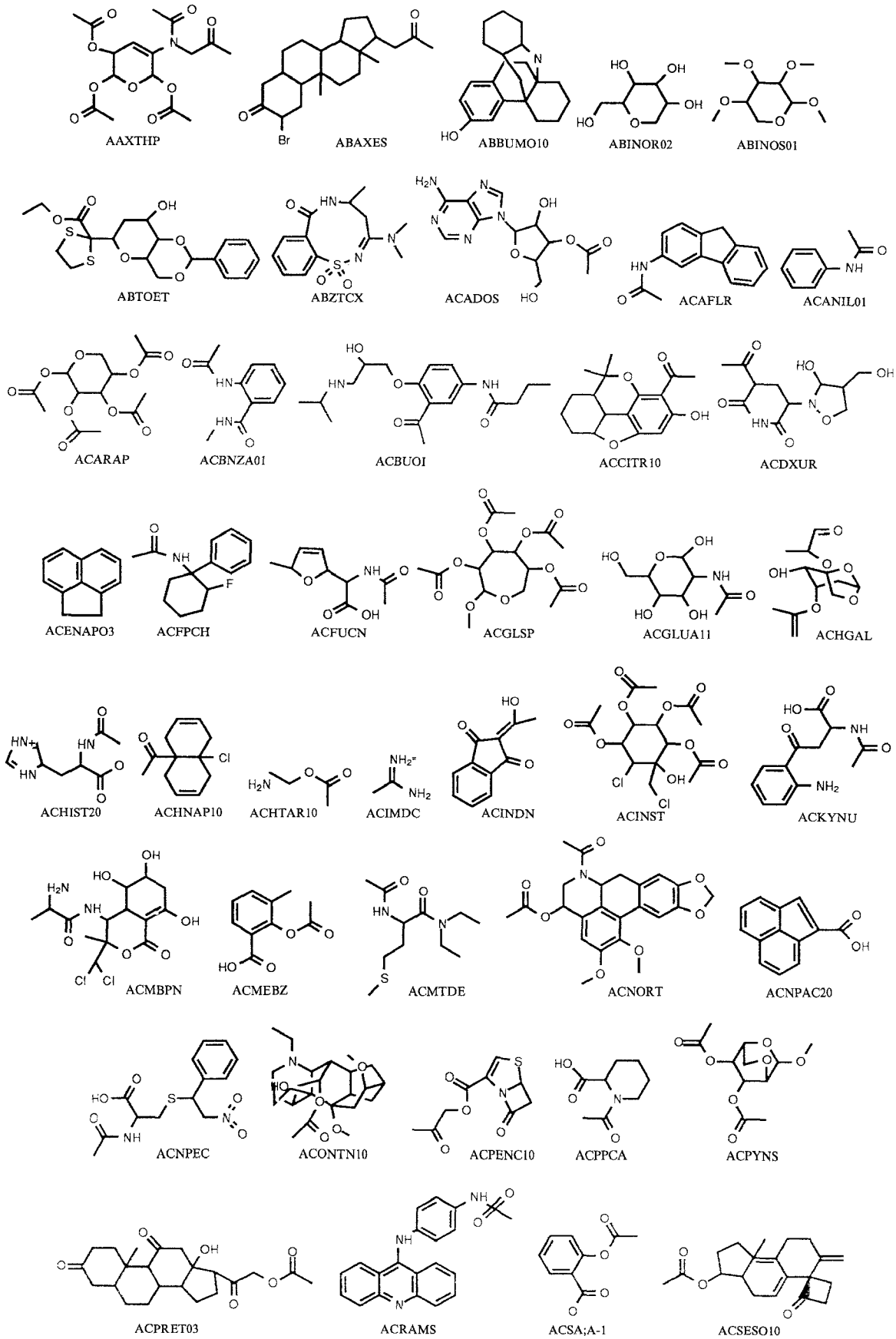

Fig. 1. Structures of the 74 CSD compounds used in this study.

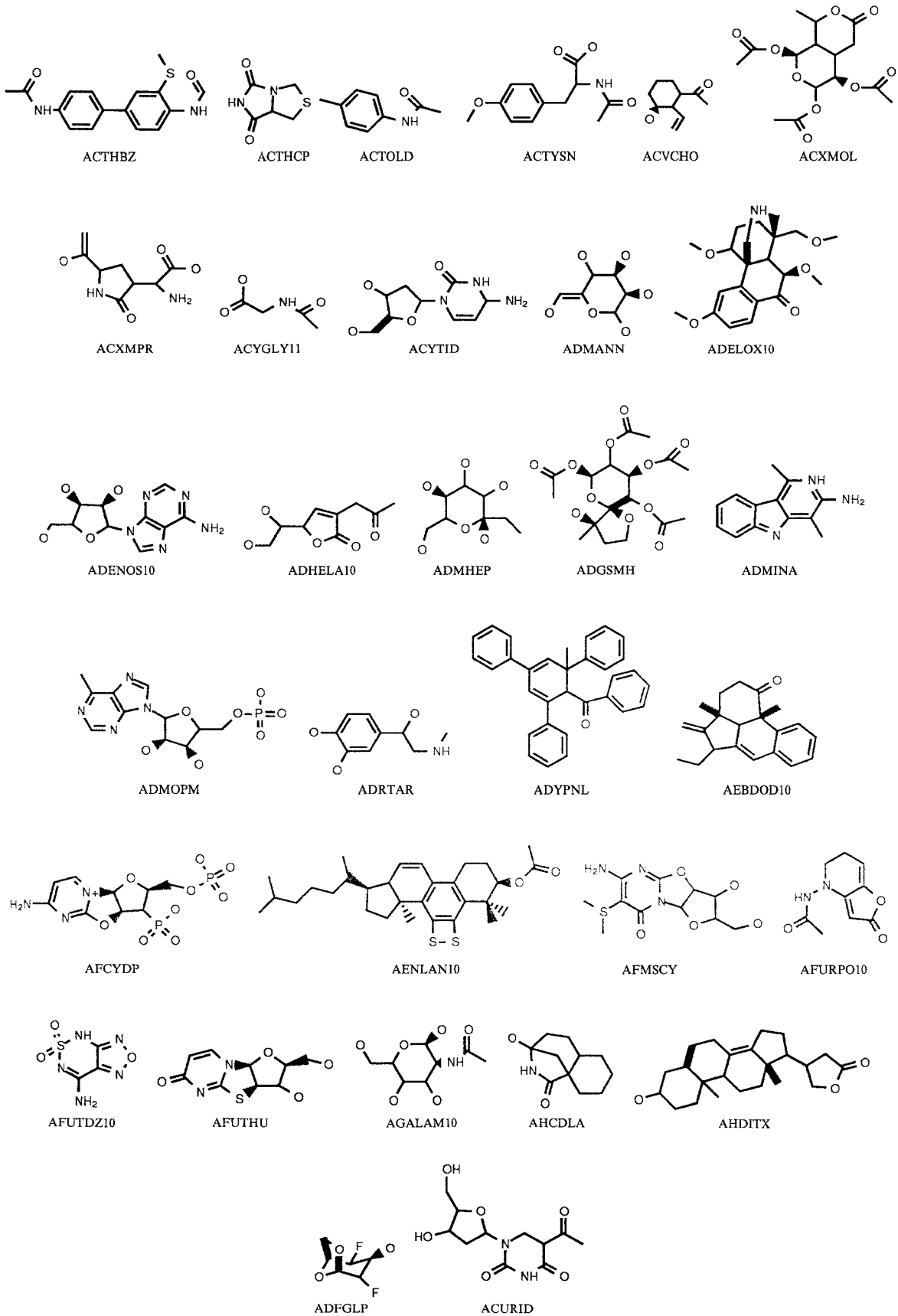

Fig. 1. (continued).

community for their use and further investigation. We have used the method as described in several 'real-life' problems. The conditions used have proved useful in all cases thus far.

The compounds used in the present study are shown in Fig. 1. They were chosen based on the fact that they have been used before for similar purposes [1] and because they represent a reasonable distribution of functional groups and structural motifs of interest to the medicinal chemist. These structures are also relatively well studied crystallographically. The structures contain from one to twelve rotatable bonds and hence span a size range of conformational analysis problems that is most frequently of interest.

These 74 molecules were all extracted from the Cambridge Structural Database (CSD). The molecules, as extracted, were found to have relatively high energies as determined by the TFF (v. 5.41) force field. Thus, they were minimized to completion in this force field. Very little change in the torsional conformations of the molecules was detected in this operation, but the energies dropped considerably (see Table 1). The median energy difference between the crystal and minimized crystal conformations of these molecules, as judged by the force field used, is 20.465 kcal/mol but three structures (ABZTCX, ACNORT and ACRAMS) had energy differences greater than 100 kcal/mol. The minimized crystal conformations were taken as the starting point of this study. All acyclic rotatable bonds (except those leading to terminal methyl groups) were selected for analysis.

The underlying assumption in the choice of these molecules and conformations was that they represented minima that were close to the lowest energy minimum for each molecule. This assumption is based on the argument

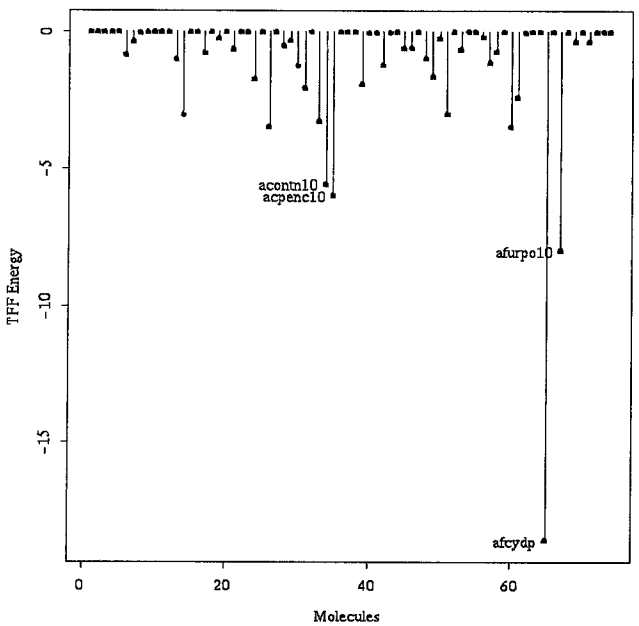

Fig. 2. Energy differences between the CEDD run1 and minimized crystal conformations.

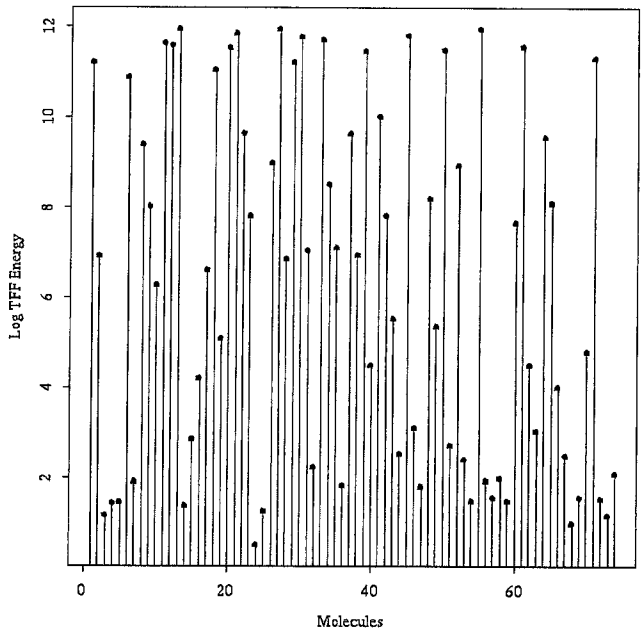

Fig. 3. Energies of the run2 zeroed-torsions starting conformations.

that, in order to crystallize in a given conformation under 'equilibrium' conditions, the particular conformation must exist in appreciable populations in the supernatant. This implies that the particular conformation is of 'low' energy. Admittedly, this assumption is a debatable one. In practice, however, none of the methods that we have applied so far to examine the conformational space of any of these molecules has not yielded any conformations considerably lower in energy (i.e., greater than, say, 15 kcal/mol lower in energy) than the minimized crystal conformation [1].

Each of the 74 minimized structures was submitted as a starting conformation to the CEDD analysis procedure. The resulting conformation for each structure was energetically analyzed with the TFF force field and with AM1 [6] single point calculations. A second set of starting conformations was generated by setting the torsion angle around every rotatable bond to a value of zero. This second set of conformations was also submitted to the CEDD method and TFF energy analysis. The energetic data is reported in Table 1.

Each of the molecules was extracted from the CSD into SYBYL (v. 5.41). Duplicate structures were removed, as were any solvent molecules, etc. Atom and bond types were checked and modified where necessary, so that they corresponded to appropriate SYBYL atom and bond types for the minimization. Each molecule was minimized in the TFF until it met a convergence criterion of ΔE = 0.01 kcal/mol. Then all acyclic bonds that did not lead to terminal symmetric groups (e.g. methyl) were identified as rotatable. Throughout this study, all of the rotatable bonds defined in this way were examined in steps of 10°. All of the results were obtained interactively on a Sun SPARCserver 490.

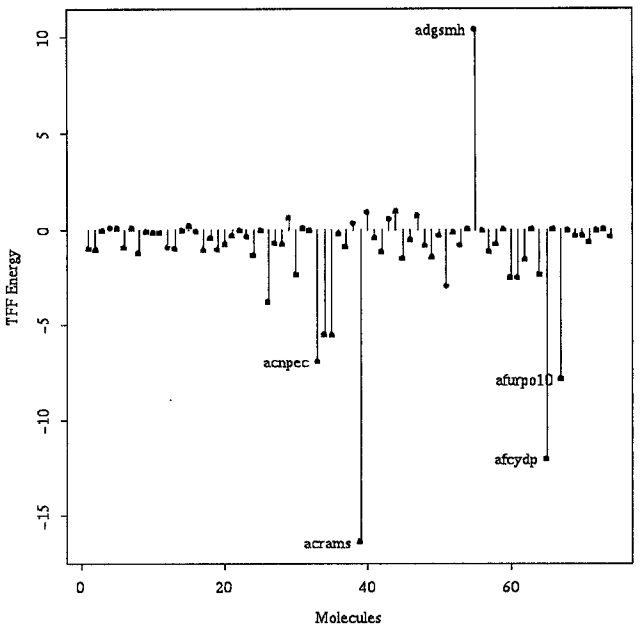

Fig. 4. Energy differences between CEDD run2 and minimized crystal conformations.

Results

As shown in Fig. 2, the minimized crystal conformations taken as starting structures were the lowest energy conformations found in virtually every case. In only four cases did CEDD find conformations whose energies were more than 5 kcal/mol lower than the minimized crystal conformation energy. There were no cases where conformations were found that were higher in energy than the starting conformation. This is exactly what had been expected. In other words, the CEDD was able to identify good low-energy conformations every time these were provided and therefore this experiment provided a good verification of the method. This was an important first step in the calibration of the method, since its utility is based on the assumption that if it was provided with reasonable structures it would recognize them as such.

The identification of good low-energy conformations was a necessary but not sufficient condition for the method to be judged useful. The second, and perhaps more important, measure of success for the CEDD method was the ability to take a poor starting conformation and to subsequently find a better one. The method should be able to traverse barriers to find lower energy structures before its true worth could be recognized.

To test this second criterion for success of the CEDD method, all of the identified rotatable bonds of each molecule were set to zero. This operation generated conformations that were quite strained, as expected. (See Fig. 3. Note the logarithmic energy scale.) Each of these high-energy conformations was then run through the CEDD. The expectation here was to find, in all cases, conformations which were considerably lower in energy than the starting one. In Fig. 4, these final CEDD energies are compared to the minimized crystal conformers. It can be seen that in most cases the arbitrarily chosen starting conformer caused the method to find conformers that were much better than the starting strained ones and whose energies were very close to those of the observed minimized crystal conformations. In only one case, ADGSMH, did CEDD settle on a conformer whose energy was more than 5 kcal/mol greater than the minimized crystal conformer energy. Thus, the method could identify good conformations and return them unchanged; it could also identify strained ones and modify them to generate conformations that were reasonably low in energy.

Finally, an independent check of the energetic conclusions was needed in order to exclude the possibility of bias introduced by the TFF. For this purpose, and in order to maintain consistency with conformational analysis methods calibration work that has been previously reported [1], the AM1 method was used to recalculate the heat of formation of the minimized crystal conformation and the conformation resulting from exposing this minimized crystal structure to the CEDD method. These values are included in Table 1. It can be seen that conclusions drawn based on the AM1 calculations do indeed closely parallel those that are reported based on the TFF energies.

The influence of computed charges on the energetic preference for different conformations is a topic of some debate in the literature. Having shown that, in the absence of any charges, the CEDD method can recognize 'good' and 'bad' conformations for a variety of small lipophilic drug-like molecules, we found it of interest to examine what, if any, would be the effect of including the

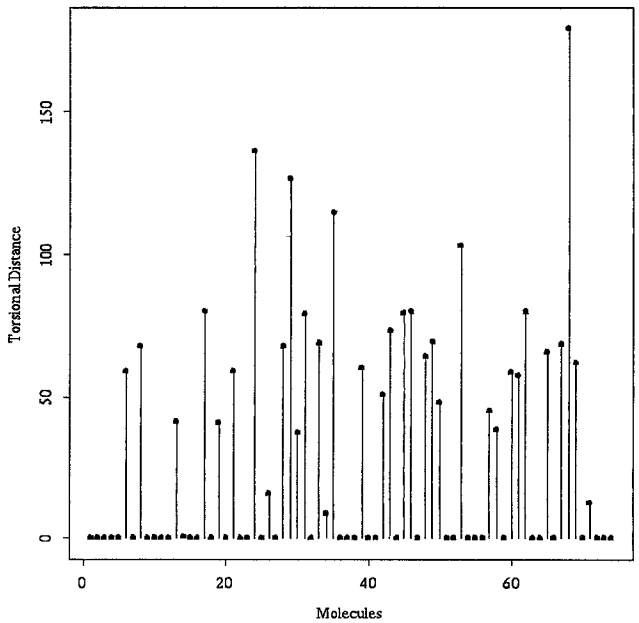

Fig. 5. Torsional distances between minimized crystal and CEDD run1 structures.

TABLE 2
TORSIONAL DISTANCE COMPARISONS

Molecule	Min. crystal vs. run1 min.	Min. crystal vs. run2 min.	Run2 initial vs. run2 min.	Molecule	Min. crystal vs. run1 min.	Min. crystal vs. run2 min.	Run2 initial vs. run2 min.
AAXTHP	0.09	91.34	122.61	ACRAMS	60.29	35.27	90.00
ABAXES	0.07	66.34	137.08	ACSALA01	0.22	92.22	106.49
ABBUMO10	0.00	3.00	0.00	ACSESO10	0.10	73.12	180.00
ABINOR02	0.19	103.29	180.00	ACTAND	50.92	59.98	113.84
ABINOS01	0.12	61.46	180.00	ACTHBZ	73.36	126.99	122.96
ABTOET	58.92	60.40	164.30	ACTOLD	0.00	130.81	50.91
ABZTCX	0.00	10.70	180.00	ACTYSN	79.85	92.31	114.65
ACADOS	67.92	81.99	149.05	ACURID	80.41	110.36	164.19
ACAFLR	0.07	5.59	163.00	ACVCHO	0.10	49.04	142.87
ACANIL01	0.07	4.28	129.80	ACXMOL	64.53	63.67	146.68
ACARAP	0.07	9.23	144.01	ACXMPR	69.69	95.71	152.74
ACBNZA01	0.19	127.57	147.37	ACYGLY11	48.30	81.97	144.00
ACBUOL	41.26	81.29	127.28	ACYTID	0.11	83.62	156.23
ACCITR10	0.51	1.56	127.28	ADELOX10	0.09	3.85	146.68
ACDXUR	0.17	49.59	139.43	ADENOS10	103.32	121.44	164.32
ACFPCH	0.08	4.08	159.65	ADFGLP	0.20	8.90	72.00
ACFUCN	80.36	101.16	146.68	ADGSMH	0.05	61.66	129.80
ACGLSP	0.07	45.45	154.14	ADHELA10	0.13	52.62	143.10
ACGLUA11	41.05	63.14	159.84	ADMANN	45.05	68.46	152.74
ACHGAL	0.06	68.59	146.68	ADMHEP	38.78	71.67	170.29
ACHIST20	58.91	116.95	137.08	ADMINA	0.10	2.70	180.00
ACHNAP10	0.07	0.00	180.00	ADMINA	0.10	2.70	180.00
ACHTAR10	0.09	53.07	180.00	ADMOPM	58.64	67.06	126.92
ACIMDC	136.53	136.04	137.08	ADRTAR	57.70	58.50	111.38
ACINDN	0.10	1.11	127.28	ADYPNL	80.23	96.71	82.09
ACINST	15.80	26.49	139.43	AEBDOD10	0.00	3.30	72.00
ACKYNU	0.17	78.69	157.95	AENLAN10	0.08	18.75	158.68
ACMBPN	67.92	47.17	114.65	AFCYDP	65.87	36.25	122.61
ACMEBZ	127.00	144.98	106.49	AFMSCY	0.09	57.90	151.03
ACMTDE	37.60	106.48	141.48	AFURPO10	68.80	98.50	108.00
ACNORT	79.38	5.38	122.60	AFUTDZ10	179.40	179.10	0.00
ACNPAC10	0.07	0.81	180.00	AFUTHU	62.02	64.35	152.74
ACNPEC	68.98	92.87	117.58	AGALAM10	0.13	63.31	163.85
ACONTN10	8.63	47.41	144.00	AGLUAM10	12.48	82.66	148.98
ACPENC10	115.06	114.18	159.99	AHARFU	0.12	71.89	152.70
ACPPCA	0.18	16.58	146.97	AHCDLA	0.00	1.50	180.00
ACPRET03	0.04	47.88	117.21	AHDITX	0.00	86.16	137.08
ACPYNS	0.12	4.30	126.77				

charges in the above runs. AM1 single point calculations had been run on the minimized crystal conformations of all molecules. Mulliken population analysis of the AM1 results yielded point charges for each of the atoms. The same set of charges was used in both sets of reruns, i.e., where the starting conformation was the minimized crystal conformation and where it was the zeroed conformation. The energy results from rerunning the entire study with charges included, as mentioned above, are collected in Table 1 and identified as 'run3' and 'run4', respectively. It can be seen that there is very little change in the overall conclusion when charges are included. Perhaps this is a consequence of the nature of the molecules chosen.

Discussion

It is important to point out that our implementation of the method does not handle ring bonds. This limits the application of this method to those problems where acyclic systems are being considered. If rings are part of the molecule being studied then suitable (and possibly multiple) conformations of the rings must be used as starting points. These ring conformations will not be modified during the run in the current version of this program.

In some of the cases (30 out of 74) the energy of the CEDD conformation that was found starting from the minimized crystal conformation was slightly lower than that of the starting one. However, these differences are slight (often less than 1 kcal/mol) and are therefore not thought to be of any consequence. To examine the possibility that different conformations of the molecule with the same energies had been found, the torsional distances [7] between the starting minimized crystal structures and those found by the CEDD method were computed. The torsional distance is calculated by representing two conformations as two ordered sets of torsional angles. These

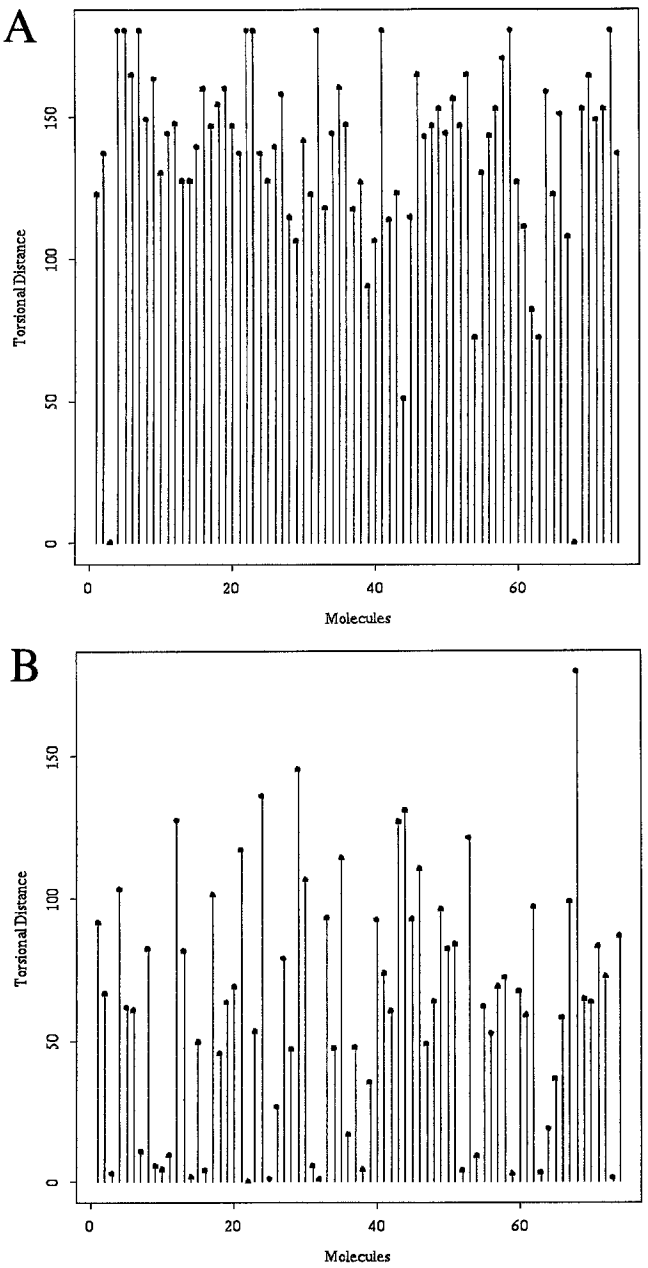

Fig. 6. Torsional distances between (A) zeroed-torsions and CEDD run2 structures; and (B) minimized crystal and CEDD run2 structures.

values are all represented as positive numbers. Then the differences between corresponding pairs of torsions are squared and summed. The square root of the sum represents the so-called torsional distance. The torsional distance comparison results are shown in Table 2. Figure 5 illustrates that, in the majority of the cases when starting with the minimized crystal conformation (run1), CEDD does not find a lower energy conformer and hence returns essentially the same conformation with a minimal torsional distance. In a significant number of cases, however, the method did indeed find conformations whose shape and energy were different from those in the starting crystal conformation. We interpret this as a further indication that, *given the force field used*, if a lower energy conformation can be found by altering torsions then this method will find it. On the other hand, when starting with alternate conformations (run2) which have large torsional distance values (Fig. 6A), CEDD modifies the conformations significantly and finds conformations that are energetically equivalent (Fig. 4) but torsionally different (Fig. 6B) from the minimized crystal conformations. It can be concluded that, when starting with the crystal 'answer', CEDD will only occasionally improve upon it, but when starting with poor conformations CEDD will find energetically good conformations that are not necessarily torsionally equivalent to the crystal answer.

We wondered if the fact that we were *recycling* through the rotatable bonds until 'self consistency' was obtained could lead to a *non* dependance on the order of the rotatable bonds. To study this possibility we have rerun one of the test cases a number of times, starting from the *same* conformation but specifying the rotatable bonds in three very different ways. AAXTHP was rerun three times, with the order of specifying the rotatable bonds being changed each time: (i) going all the way out on each of the side chains and going round the central ring clockwise; (ii) going all the way out on each side chain but then taking the side chain 'opposite' the ring as the next choice; and (iii) specifying all of the rotatable bonds closest to the ring first, then the next ones out and so on. In each case the starting conformation was the X-ray one. The result from each of these runs was identical.

Our explanation for this is *not* that we are performing the equivalent of a global minimum search, because clearly we are not. Rather, the *re-iteration* over all of the rotatable bonds until some level of 'self consistency' is reached means that this method removes the dependance of the final answer on the order of selection of the rotatable bonds. We use this method routinely in our laboratories and these results are completely typical.

From an examination of the four runs described for each of the molecules (run1 through run4) it can be seen that the method returns structures that are close in energy to the minimized crystal conformation, regardless of whether the starting conformation is that found in the X-ray analysis or that which has all of its torsions zeroed. No great dependance on the presence of charge appears to occur, either. It is important to point out that the conformational energies used for the comparisons in the runs where charges were used have all been recalculated on the final conformations after removing the charges. This was done in order to make these numbers directly comparable to those of the previous runs.

Conclusions

From the data presented above we conclude that the CEDD method described above works well in the cases

that it has been tested against. This gives us confidence to use it in cases where the answers are not known ahead of time.

The utility of the method derives from its ability to quickly answer the question of whether a given conformation of a molecule is energetically 'reasonable', without having to subject the molecule to exhaustive techniques. As such, it forms a useful complement to such techniques for cases where there is no desire to exhaustively enumerate all of the so-called low-energy conformations. The method scales in a roughly linear fashion with the number of rotatable bonds considered and so may be applied to larger problems. Problems involving about 10 rotatable bonds, for example, can be solved interactively. The method has already been used in our laboratories on several practical drug design problems and its utility is expected to grow.

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5 This set of molecules has been used as a test set for the validation of several force fields and is the subject of a systematic analysis of conformational analysis methods in our laboratories. See Ref. 1 above and references cited therein.

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Appendix

SPL code for the algorithm

```
C E D D -- CONFORMATIONAL ENERGY DOWNWARD DRIVER

@MACRO
cedd sybylbasic yes
set cgq 0
# STEP1: Take as input the rotatable bond, the start and finish
# angles and the step size.
setvar root_name %prompt(string "myfile" "enter the base name for this run"\
      "any base name to be used for the family of files generated here")
setvar numrotbnd %prompt(INT "1" "number of rotatable bnds to be \
      defined?" "any number of available bonds")
setvar nsteps %prompt(int "10" "number of steps" "any integer")
setvar incr %math(360 / "$nsteps")
setvar molarea %prompt(M_SEL "M1" "molecule area to use" "area where \
      molecule is.")
setvar minenergy " "
setvar mintor 0
setvar torval 0
setvar loopcnt 0
setvar keep_going "TRUE"
for i in %range(1 $numrotbnd)
   echo %cat("Enter the 4 atoms which define rotatable bond" $i)
   setvar %cat("atm1" "$i") %prompt("A_SEL" "1" "pick first atom." \
         "any atom in the molecule.")
   setvar %cat("atm2" "$i") %prompt("A_SEL" "2" "pick second atom." \
         "any atom in the molecule.")
   setvar %cat("atm3" "$i") %prompt("A_SEL" "3" "pick third atom." \
         "any atom in the molecule.")
   setvar %cat("atm4" "$i") %prompt("A_SEL" "4" "pick fourth atom." \
         "any atom in the molecule.")
   setvar ba1 %eval(%cat("$" "atm2" "$i"))
   setvar ba2 %eval(%cat("$" "atm3" "$i"))
   setvar bondexpr %cat(%atom_info($ba1 id) "=" %atom_info($ba2 id))
   color bond $bondexpr violet
endfor
# STEP2: Save the input structure and initial energy.
energy $molarea done >$nulldev
setvar minenergy $ENERGY_TOTAL
modify molecule name $molarea $ENERGY_TOTAL
mol2 out $molarea %cat("$root_name" "_ini.mol2")
# STEP3: Increment the first rotatable bond through the whole range
# by the step size
while %streql($keep_going TRUE)
   setvar keep_going "FALSE"
   for i in %range(1 $numrotbnd)
      measure torsion    %eval(%cat("$" "atm1" "$i")) \
                         %eval(%cat("$" "atm2" "$i")) \
                         %eval(%cat("$" "atm3" "$i")) \
                         %eval(%cat("$" "atm4" "$i"))
      setvar torval $MEASURE_TORSION
      setvar mintor $torval
      for j in %range(1 $nsteps)
         setvar torval %math($torval + $incr)
         modify torsion  %eval(%cat("$" "atm1" "$i")) \
                         %eval(%cat("$" "atm2" "$i")) \
                         %eval(%cat("$" "atm3" "$i")) \
                         %eval(%cat("$" "atm4" "$i")) $torval >$nulldev
#     STEP4: At each step measure energy
#     Find the value for the lowest E
#     Set the bond"s tor to the value for the lowest E.
      energy $molarea done >$nulldev
         if %lt($ENERGY_TOTAL $minenergy)
            setvar minenergy $ENERGY_TOTAL
            setvar mintor $torval
            setvar keep_going "TRUE"
         endif
      endfor
      modify torsion  %eval(%cat("$" "atm1" "$i")) \
                      %eval(%cat("$" "atm2" "$i")) \
                      %eval(%cat("$" "atm3" "$i")) \
                      %eval(%cat("$" "atm4" "$i")) $mintor
# STEP7: Loop to the next bond and repeat from step3 to here.
   endfor
# STEP8: When all bonds are done check E of the FINAL structure.
   energy $molarea done >$nulldev
# STEP9: Have any torsions been modified? If yes loop again.
# STEP10: If at least one tor is different then repeat from step2.
   setvar loopcnt %math($loopcnt + 1)
endwhile
# STEP11: Report the final structure and energy.
energy $molarea done >$nulldev
modify mol name $molarea $ENERGY_TOTAL
mol2 out $molarea %cat("$root_name" "_min.mol2")
echo %cat("CEDD looped " $loopcnt " times."
```