Notes

Optimizing the Binding of Fullerene Inhibitors of the HIV-1 Protease through Predicted Increases in Hydrophobic Desolvation[||]

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We have developed and applied a computational strategy to increase the affinity of fullerene-based inhibitors of the HIV protease. The result is a ∼50-fold increase in affinity from previously tested fullerene compounds. The strategy is based on the design of derivatives which may potentially increase hydrophobic desolvation upon complex formation, followed by the docking of the hypothetical derivatives into the HIV protease active site and assessment of the model complexes so formed. The model complexes are generated by the program DOCK and then analyzed for desolvated hydrophobic surface. The amount of hydrophobic surface desolvated was compared with a previously tested compound, and if this amount was significantly greater, it was selected as a target. Using this approach, two targets were identified and synthesized, using two different synthetic approaches: a diphenyl C_{60} alcohol (**5**) based on a cyclopropyl derivative of Bingel (*Chem. Ber.* **1993**, *126,* 1957−1959) and a diisopropyl cyclohexyl C_{60} alcohol (**4a**) as synthesized by Ganapathi et al. (*J. Org. Chem.* **1995**, *60,* 2954−2955). Both showed tighter binding than the originally tested compound (diphenethylaminosuccinate methano-C_{60}, $K_i = 5\ \mu$M) with K_i values of 103 and 150 nM, respectively. In addition to demonstrating the utility of this approach, it shows that simple modification of fullerenes can result in high-affinity ligands of the HIV protease, for which they are highly complementary in structure and chemical nature.

Introduction

For approximately the last 10 years, the human immunodeficiency virus protease (HIVP) has been a target for anti-HIV drug development.[1] We were drawn to the fullerene core as the basis for inhibitors of this enzyme because the fullerene structure of C_{60} is highly complementary, both sterically and chemically, to HIVP, thus potentially leading to its binding to the HIVP active site.[2,3] The active site of uncomplexed HIVP is approximately cylindrical and lined predominantly with hydrophobic amino acids.[4] Our original modeling suggested that an appropriately derivatized C_{60} would fit tightly in the active site.[2] Furthermore, the modeled complex indicated that the majority of the surface that becomes desolvated upon complexation would be hydrophobic. An estimate of the binding affinity of a fullerene based on the calculated hydrophobic desolvation indicated a potential affinity constant approximately in the low-micromolar to nanomolar range. This was confirmed with experiment, and compound **1** (Table 1) was shown to bind with a K_i value of 5 μM.[2] The modeled complex of this tested compound, generated using the program DOCK, showed the core fullerene moiety fitting snugly into the active site, with the water-solubilizing "arms" extending out of the active site into the solvent (Figure 1a,b). This modeled complex was supported in several ways: (1) The binding kinetics were competitive. (2) The binding affinity of a closely related derivative, the diamine parent of compound **1,** had a nearly identical K_i value; this insensitivity of the binding affinity to the nature of the solubilizing arms supported minimal interaction of them with the active site. (3) The estimated binding affinity was consistent with the amount of desolvated hydrophobic surface.

Because of the success of the initial model design and consistency of the model with experimental data, we have sought to improve on the binding affinity of these compounds in a rational manner by the use of modeling techniques to introduce addends on the C_{60} surface that are able to exploit regions of the HIVP which did not interact with the initial lead compounds. The method involves identification of regions unexploited in model complexes of the previously tested compounds. This is followed by design of hypothetical derivatives which are potentially able to interact with these regions. The hypothetical derivatives are then fitted into the active site, using the program DOCK.[5] The modeled com-

[||] Abbreviations: HIV (human immunodeficiency virus), HIVP (human immunodeficiency virus protease), NOESY (nuclear Overhauser and exchange spectroscopy), TROESY (transverse rotating frame Overhauser enhancement spectroscopy), DIBAL-H (diisobutylaluminum hydride), NMP (*N*-methylpyrolidone), DMSO (dimethyl sulfoxide), EDTA (ethylenediamine tetraacetate), HMPA (hexamethylphosphorus triamide), LDA (lithium diisopropylamide), TFA (trifluoroacetic acid).

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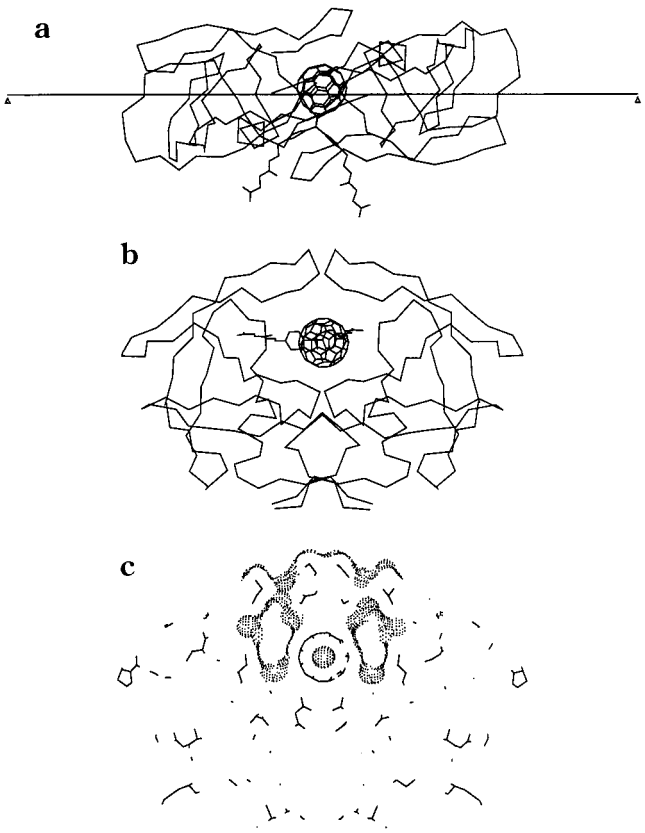


Figure 1. (a) DOCK-generated complex of compound **1** with HIVP in "top" view. The thin line in panel a indicates the plane of the cross section depicted in panel c. (b) This same complex viewed from the "front". (c) Identical front view of the compound **1** complex, but with only a thin cross section of the complex showing. The cross section contains the C_2-axis and both catalytic aspartates. The dots are the points of a solvent-accessible molecular surface generated from the complex.

plexes are then analyzed for the amount of hydrophobic surface desolvation brought about through complex formation. If this amount is a significant increase over the hydrophobic surface desolvation achieved by the lead compound **1**, it is selected as a synthetic target. Because the shape of C_{60} is highly complementary to the HIVP binding site while being a completely conformationally restricted scaffold, the modeling of hypothetical compounds and their interactions with the HIVP active site are simplified.

Modeling Strategy

The first step in the design strategy was the identification of regions of the HIVP that were unable to interact with the previously tested compound **1**. Examination of modeled complexes of compound **1** with the HIVP and their associated molecular surfaces indicated large apolar regions in the middle of the active site with which compound **1** was unable to interact. Figure 1c shows a thin cross section through the middle of the DOCK-generated model complex (i.e., containing the C_2-axis and catalytic aspartates) of compound **1** and the protease. It shows two symmetrical water-exposed channels lined predominantly with nonpolar surfaces. These channels are present because the active site is not a perfect cylinder but instead is elliptical in cross section, and the bulkiness of the side chains of **1** prevents them from gaining access to these areas. The goal was to design C_{60} derivatives that could fill one or both of these regions with a nonpolar group and, in so doing, increase the amount of hydrophobic desolvation relative to compound **1** and therefore increase the binding affinity.

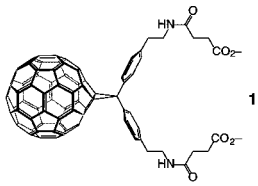
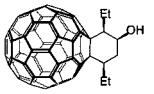
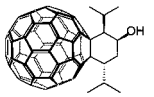
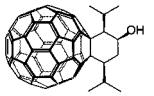
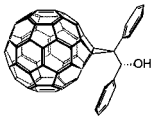
The structure of hypothetical derivatives were modeled using the SYBYL package.[6] The program MINDOCK was subsequently used to generate hundreds of possible orientations of the hypothetical derivative with the active site. MINDOCK is a version of DOCK which uses rigid body minimization to relieve unfavorable contacts within the complexes.[7] The complexes were then scored within MINDOCK using a previously described approach which sums "good" and "bad" van der Waals interactions and electrostatics.[5]

Following the initial screen of the compounds using DOCK's scoring, top scoring complexes from each run were further analyzed. Specifically, the amount of nonpolar surface that was desolvated during complex formation was analyzed. Molecular surfaces for the inhibitor alone, the uncomplexed HIVP, and the inhibitor/protease complex were generated using the program MS.[8] MS generates surfaces which form at the contact point between a probe water sphere and the van der Waals surface of the molecule being analyzed. The files that are generated contain atom types for each surface element. Using these files and an 'awk' script, the total surface for each atom type N, O, and C was separately summed. The total of C atom areas was used as an estimate of nonpolar surface. Using the difference between the final complex surface and the sum of the uncomplexed surfaces, it was possible to determine the amount of surface desolvated and the proportion of it due to nonpolar desolvation. Typically, the desolvation due to nonpolar surface was in the 90% range. The speed with which MINDOCK generated complexes allowed the procedure to be used iteratively, adding or removing atoms to a derivative to optimize the increase in hydrophobic desolvation.

When we analyzed the originally tested compound **1**,[2] we found that it formed a complex that could desolvate 333−352 Å^2 in nonpolar surface depending on slight variations in the geometry of the complex (Table 1). The diamine parent of compound **1** created similar complexes (i.e., with the solubilizing arms extending into solvent) and desolvated 381 Å^2 of nonpolar surface. The value of 381 Å^2 became a floor upon which hypothetical compounds would have to improve substantially in order to be selected as synthetic candidates. Table 1 shows some of the 40 compounds analyzed using this method, together with their desolvated surface area breakdowns. The amount of increase in hydrophobic desolvation relative to compound **1** was used to estimate the potential improvement in binding relative to this compound.

Many of the analyzed compounds are chiral and are most readily synthesized as racemates. The modeling required analysis of each enantiomer separately, because the different enantiomers should interact differently with the chiral environment of the protease active site. In Table 1, therefore, the surface desolvation listed is for the enantiomer that gave the largest amount of desolvation, which is the actual enantiomer pictured. Figure 2c shows the cross section of HIVP, now binding

Table 1. Fullerene Derivatives That Were Analyzed for Their Ability To Desolvate Nonpolar Surfaces in Model Complexes Formed with the HIV-1 Protease[a]

Compound		Hydrophobic Desolvation (Å²)	K_i (s.e.)
	**1**	–332.8, –352.1	5μM (0.98)
	**2**	–367.6	
	**3**	–457.8	
	**4a**	–444.7[b]	150nM (48)
	**4b**	–485.4	
	**5**	–472.4	103nM (37)

[a] Molecules **2** and **3** were not synthesized but are shown to represent some of the range of derivatives that were analyzed and rejected as possible synthetic targets. s.e.: standard error. [b] Several conformations of this compound were originally used for docking in selecting it as a synthetic target, all of them chair conformations. The major experimentally observed conformation is a boat, however. The hydrophobic desolvation indicated is the value of desolvation for this boat structure, a value that is somewhat smaller than the originally determined value but still a large enough improvement over compound **1** to warrant testing.

compound **5** in a DOCK-generated complex. The previously water-exposed channel (Figure 1c) is now partially occluded by the substituent on the fullerene framework, producing a decrease in nonpolar surface exposure to water. This is supported by the total amount of nonpolar surface desolvation for this compound indicated in Table 1, which is 472 Å², an improvement of ~90 Å² over compound **1**. A substantial increase in hydrophobic desolvation (445 Å² total) is also calculated for the cyclohexane-fused C_{60} derivative **4a**.[9] Its diastereomer **4b** with an all-cis configuration of the substituents on the cyclohexane ring fits even better based on these criteria (Table 1), but its synthesis has so far proven challenging (see below). One of a number of possible bis-adduct derivatives,[10] diammonium salt **3**, was also calculated to give a large increase in hydrophobic desolvation (458 Å² total). This example illustrates the potential of multiple adducts of fullerenes, although their synthesis in a straightforward fashion needs to be developed, possibly through a combinatorial approach.[11,12]

Results and Discussion

Compounds **4a**,**b** and **5** were selected as targets for synthesis and testing because of the relatively large

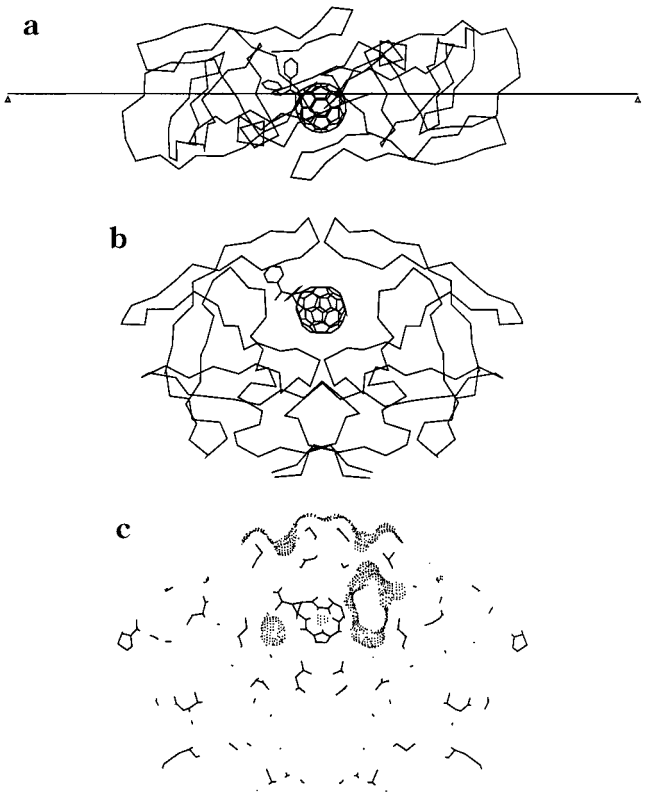
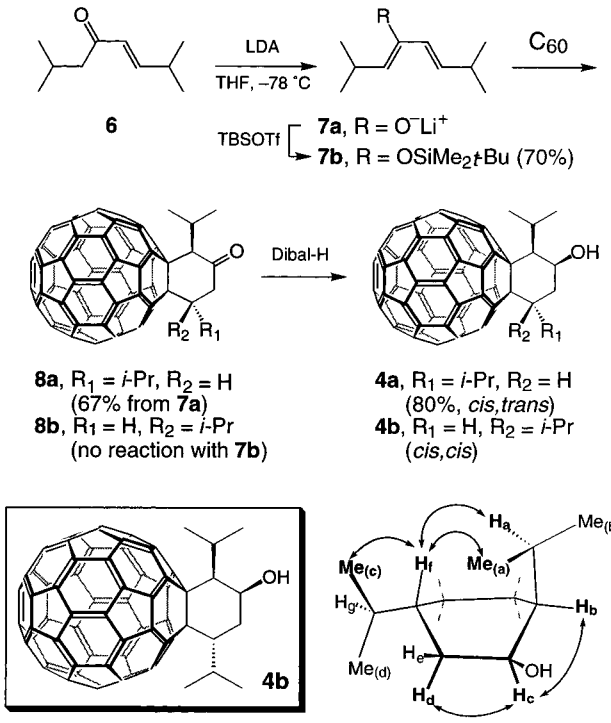


Figure 2. (a) DOCK-generated complex of compound **5** with HIVP in "top" view. The thin line in panel a indicates the plane of the cross section depicted in panel c. (b) This same complex viewed from the "front". (c) Identical front view of the compound **5** complex, but with only a thin cross section of the complex showing. The cross section contains the C_2-axis and both catalytic aspartates. The dots are the points of a solvent-accessible molecular surface generated from the complex.

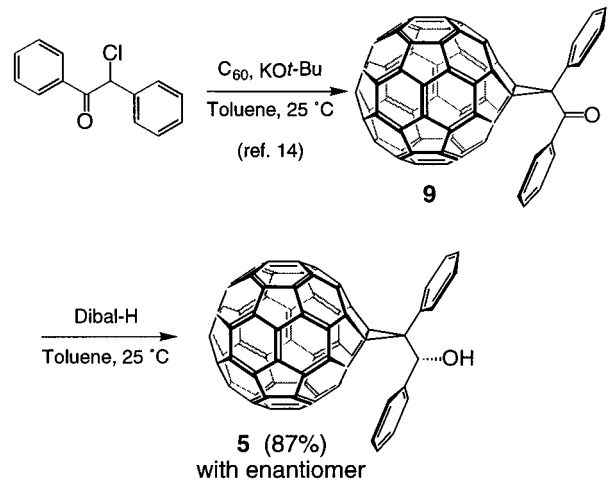
increases in hydrophobic solvation they produced, as well as for their relative ease of synthesis. Our initial interest was in bifunctional compounds such as compound **3** which potentially could interact with both solvent-exposed channels simultaneously. For synthetic ease, however,[10] monofunctional compounds were first examined closely and were found to satisfy the design criteria, i.e., a large increase in hydrophobic desolvation relative to compound **1**.

The preparation of compound **4b** was attempted first as described in a preliminary communication because of its large hydrophobic desolvation determined by calculation (Table 1).[9] A Diels–Alder approach to prepare the ketone **8b** with cis geometry of the isopropyl substituents was investigated (Scheme 1). Not unexpectedly, the Diels–Alder reactivity of the diene **7b** was found to be too low to undergo cycloaddition with C_{60}. Accordingly, the desired cycloadduct **8b** could not be obtained under various conditions (toluene, 110 °C; 1,2-dichlorobenzene, up to 180 °C; toluene, sonication, 50 °C).[13] To circumvent this impass, we utilized a stepwise cyclization reaction of the lithium dienolate **7a** (**6**, LDA, THF, −78 °C) to C_{60} which proceeds via a double-Michael addition mechanism.[14] The diastereomerically pure ketone obtained in this reaction was subsequently identified as the trans diastereomer (**8a**) by unequivocal stereochemical assignment of its alcohol reduction product **4a** from 2D NOESY and 2D T-ROESY ^{1}H NMR experiments (Supporting Information).[15] Both sets of

Scheme 1



Scheme 2



2D experiments showed key correlations between the flagpole hydrogen H_f and H_a, $Me_{(a)}$, and $Me_{(c)}$ and between H_c and H_b, H_d, which taken with the H−H coupling data demonstrate that alcohol **4a** exists largely in the boat conformation in solution.

Compound **5** was prepared by DIBAL-H reduction of the ketone **9** whose synthesis has been reported by Bingel (Scheme 2).[16] The original intent behind the OH groups on compounds **4a,b** and **5** was to use them as branch points to introduce additional functionalities, primarily to aid in solubilization and possibly aid in binding. Top scoring complexes of compounds **4a** and **5,** however, show the OH group partially buried in the active site, so that additional groups could not be linked through the OH without introducing severe steric clashes within the active site. The modeled complex of the alcohol **4b,** however, positions the OH group in contact with solvent and should allow further derivatization without abrogating the specific binding mode modeled. Further derivatization of the OH groups in **4a** and **5** could produce a molecule which binds in a different orientation, but with equal or even increased hydrophobic desolvation and affinity. We are currently exploring this possibility.

Fortunately, both of the compounds **4a** and **5** were soluble enough to assay in the presence of 1% (v/v) organic solvent. *N*-Methylpyrrolidone (NMP) was used in the case of **4a** and dimethyl sulfoxide (DMSO) in the case of **5**. The results are shown in Table 1. Compound **4a** has a K_i of 150 nM and compound **5** a K_i of 103 nM, representing a significant increase in affinity of ~50-fold relative to the lead compound **1**. The assay conditions used were identical to those originally used for compound **1**[2] (50 mM NaOAc, pH 5.5, 1.0 M NaCl, 5% glycerol, 1% NMP or DMSO, respectively, 2 mM EDTA), except that the relatively stable mutant enzyme Q7K was used.[17] A control experiment showed that the K_i of compound **1** with the mutant enzyme was identical with its originally determined K_i. The use of standard high-salt conditions for the assay undoubtedly contributes to the affinity of the tested compounds, which is primarily hydrophobically driven. However, of primary interest to this work is the *relative* change in affinity, in comparison to the compounds that were tested in an identical manner, i.e., compound **1**. As before, the inhibitors were preincubated with the enzyme for 5 min. We found evidence for a slow-on kinetics without preincubation; however, with a 5-min preincubation, time courses for the evolution of complex were linear with respect to time, with 0, or near 0, intercepts.

The data were best fit to a competitive model of inhibition, although the standard errors for alcohols **4a** and **5** shown in Table 1 are high. Typical standard deviation in repeated determinations of the inhibited velocities was 30% of the mean with these compounds, whereas the identical assay with a control inhibitor (acetylpepstatin) showed standard deviations of 8% from the mean. This may be due to solubility problems and possible surface absorption effects leading to some scatter in the assay results.

Conclusions

The purpose of the work described in this paper was to improve the affinity of fullerene inhibitors of the HIVP and to explore the use of docking as an iterative design tool. Compounds **4a** and **5** satisfied the design criteria of increased hydrophobic desolvation, relative to previously tested compounds, and showed significantly increased binding affinity. In their current form, these compounds are probably too nonpolar to be drugs. However, because large regions of their surfaces are both unmodified and in solvent contact in modeled complexes, there is an opportunity to add new elements to the unmodified regions that can potentially add binding energy as well as modulate solubility, toxicity,[3,18] and bioavailability. The total surface area of compounds **4a** and **5** (molecular surfaces of 466 and 485 Å^2, respectively) is directly comparable to that of typical clinically used HIVP inhibitors (e.g., Indinavir, 544. Å^2 total ms). Despite having a relatively high molecular weight, the high density of packing of the atoms in the fullerene core means that the actual surface presented to solvent, which is what determines a molecule's interactions with solvent and protein, is comparable to clinically relevant compounds.

In general the analysis of the interaction of fullerene derivatives with the HIVP active site is simplified, in part due to the nature of the fullerene sphere and its complementarity to the HIVP active site. The starting point for design is an active site in large contact with a completely conformationally restricted C_{60} sphere. Additional elements that are introduced to the C_{60} surface are positioned by the sphere, which can orient itself in a limited number of ways, thereby simplifying the prediction of the binding mode. The ability of the fullerene surface to position multiple elements required for binding to a target, and to do so in a conformationally restricted manner, may make them of utility in pharmaceutical applications as generic, conformationally restricted pharmacophore scaffolds.[19]

Experimental Section

Syntheses. 2,7-Dimethyl-5-octen-4-one (6). To a solution of diisopropylamine (5.05 g, 7 mL, 50 mmol) in 5 mL of THF cooled to 0 °C was added 25 mL (50 mmol) of a 2.0 M solution of *n*-BuLi in hexanes, and the mixture was stirred for 10 min at 25 °C. The resulting LDA solution was cooled to −78 °C, 4-methyl-2-pentanone (5.0 g, 50 mmol) in 8 mL of THF was added dropwise, and the reaction was stirred for 20 min at −78 °C. Then, isobutyraldehyde (3.6 g, 50 mmol) in 10 mL of THF was added dropwise over a period of 5 min at −78 °C. After the addition, the reaction mixture was allowed to reach 25 °C. The reaction was quenched with water and acidified with 3 M HCl and the aqueous layer extracted with ether (3 × 30 mL). The combined organic layer was washed with water, $NaHCO_3$, and brine and dried over anhydrous Na_2SO_4. The solvent was evaporated to give the crude β-hydroxy ketone.

The crude β-hydroxy ketone was heated at reflux overnight in a mixture of 20 mL of CH_2Cl_2 and 4 mL of trifluoroacetic acid. The reaction mixture was diluted with 100 mL of ether, washed with water, $NaHCO_3$, and brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue distilled in a Kugelrohr apparatus (50 °C, ∼0.5 Torr) to obtain pure enone **6** as a colorless oil (6.0 g, 78%): ^{1}H NMR (360 MHz, $CDCl_3$) δ (ppm) 0.91 (d, J = 6.7 Hz, 6H, CH_3), 1.05 (d, J = 6.9 Hz, 6H, CH_3), 2.13 (d of sept, J = 7.0, 6.9 Hz, 1H, Me_2CHCH_2), 2.39 (d, J = 7.0 Hz, 2H, CH_2), 2.42 (sept of d, J = 6.7, 1.4 Hz, 1H, Me_2CH-CH=), 6.02 (dd, J = 16.0, 1.4 Hz, 1H, HC=C*H*C=O), 6.76 (dd, J = 16.0, 6.7 Hz, 1H, *H*C=CHC=O); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ (ppm) 21.0, 22.4, 24.8, 30.8, 48.8, 127.6, 153.0, 200.4; FT-IR (film) ν (cm^{-1}) 2958 (vs), 2859 (m), 1685 (s, C=O), 1668 (s, C=O), 1630 (s), 1463 (m), 1358 (m).

4-(*tert*-Butyldimethylsilyloxy)-2,7-dimethyl-3,7-octadiene (7b). LDA was generated as above at 0 °C from diisopropylamine (216 mg, 0.3 mL, 2.13 mmol) and *n*-BuLi (1.6 M, 0.9 mL, 1.44 mmol) in 3 mL of THF. After addition of 1.5 mL of HMPA, the enone **6** (200 mg, 1.30 mmol) in 2 mL of THF was added dropwise at −78 °C, and the reaction was stirred for 20 min. *tert*-Butyldimethylsilyl triflate (0.35 mL, 1.55 mmol) was added, and the reaction mixture was slowly warmed to 25 °C and stirred further for 2 h. The reaction was quenched with 10 mL of water and extracted with pentane (3 × 15 mL). The combined pentane extracts were washed with water and brine and dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue purified by flash chromatography on silica gel with hexane to give enol ether **7b** as a colorless oil (245 mg, 70%): ^{1}H NMR (360 MHz, $CDCl_3$) δ (ppm) 0.12 (s, 6H, SiCH_3), 0.95 (d, J = 6.7 Hz, 6H, CH(CH_3)$_2$), 1.002 (s, 9H, C(CH_3)$_3$), 1.003 (d, J = 6.7 Hz, 6H, CH(CH_3)$_2$), 2.32 (sept of d, J = 6.7, 1.8 Hz, 1H, Me_2CH), 2.70 (d of sept, J = 9.7, 6.7 Hz, 1H, Me_2CH), 4.52 (d, J = 9.7 Hz, 1H, *H*C=C-(OTBS)), 5.7−5.8 (AB-m, 2H, *H*C=C*H*); ^{13}C NMR (90.5 MHz, $CDCl_3$) δ (ppm) −3.7, 18.5, 22.4, 23.1, 24.9, 26.0, 30.8, 121.0, 125.9, 136.2, 146.3; FT-IR (film) ν (cm^{-1}) 2948 (vs), 2865 (vs), 1619 (w), 1463 (m), 1360 (m), 1251 (s), 997 (m); HR-MS (CI, NH_3) calcd for $C_{16}H_{32}SiO$ (MH^+) 269.2301, found 269.2538.

Ketone 8a, [(*trans*-3′,6′-Diisopropyl-4′-oxo)-1,2-cyclohexano]buckminsterfullerene. LDA was generated by addition of 0.68 mL (1.0 mmol) of 1.47 M *n*-BuLi to a solution of 111 mg of *i*-Pr_2NH (0.15 mL, 1.1 mmol) in 1 mL of THF cooled to 0 °C, and the reaction was stirred for 10 min at 0 °C. A solution of enone **3a** (184.8 mg, 1.2 mmol) in 7 mL of THF was added to the LDA solution at −78 °C, and the reaction mixture was stirred further for 15−20 min. A total of 1.6 mL (∼8 equiv) of the resulting solution was added to C_{60} (16.0 mg, 0.0222 mmol) in 20 mL of toluene at −50 °C, and the reaction mixture was stirred for 10 min and quenched with 3 mL of 0.5 M HCl. The toluene layer was separated, the aqueous layer was extracted with toluene, and the combined toluene layers were washed with water and brine and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and the residue was purified by flash chromatography on silica gel. Initial elution with toluene/cyclohexane (2:8) gave 2 mg of unreacted C_{60}. Further elution with toluene afforded ketone **5a** (11.4 mg, 59%, 67% based on recovered C_{60}): ^{1}H NMR (500 MHz, $CDCl_3/CS_2$, 1:4) δ (ppm) 1.11 (d, J = 6.7 Hz, 3H, CH_3), 1.40 (d, J = 6.4 Hz, 3H, CH_3), 1.41 (d, J = 6.8 Hz, 3H, CH_3), 1.43 (d, J = 6.9 Hz, 3H, CH_3), 3.16 (dd, J = 20.0, 3.0 Hz, 1H, H_c), 3.27 (d of sept, J = 6.7, 1.5 Hz, 1H, H_f), 3.35 (d of sept, J = 6.4, 6.0 Hz, 1H, H_a), 3.40 (dd, J = 20.0, 14.5 Hz, 1H, H_d), 3.83 (d, J = 6.0 Hz, 1H, H_b), 3.98 (ddd, J = 14.5, 3.0, 1.5 Hz, 1H, H_e); ^{13}C NMR (125.7 MHz, $C_2D_2Cl_4/CS_2$, 1:3) δ (ppm) 18.6, 20.4, 24.1, 27.1, 27.2, 29.8, 32.8, 37.9, 46.7, 67.8, 68.1, 134.6, 134.7, 135.1, 135.8, 138.7, 138.8, 139.8, 140.4, 141.2 (2C's), 141.3, 141.46, 141.53, 141.54, 141.7, 141.8, 141.94 (2C's), 141.99, 142.01, 142.45, 142.46, 142.5, 142.6, 142.8, 142.9, 143.0, 143.9, 144.2, 144.3, 144.4, 144.5, 144.7, 144.84, 144.85, 145.1, 145.20 (2C's), 145.21 (2C's), 145.25, 145.46, 145.51, 145.53, 145.9, 146.05, 146.06, 146.10, 146.16, 146.21, 146.30, 146.32, 147.29, 147.33, 147.40, 153.9, 155.3, 156.3, 209.3; FT-IR (KBr) ν (cm^{-1}) 2957 (m), 1719 (s, C=O), 1460 (m), 527 (s).

Alcohol 4a, [(*cis,trans*-3′,6′-Diisopropyl-4′-hydroxy)-1,2-cyclohexano]buckminsterfullerene. A solution of ketone **8a** (55 mg, 0.063 mmol) in 40 mL of toluene was cooled to 0 °C, 65 μL (0.065 mmol) of a 1 M solution of DIBAL-H was added, and the reaction stirred for 20 min. TLC of the reaction mixture indicated the presence of large amounts of starting material. A total of 140 μL (0.140 mmol) of DIBAL-H was added in two portions, and the reaction was stirred further for 30 min. The reaction was quenched with 10 mL of water, and the toluene layer was separated. The aqueous layer was extracted with toluene (10 mL), and the combined organic phases were washed with water and brine and dried over anhydrous Na_2SO_4. Removal of the solvent and flash chromatography over silica gel with cyclohexane/toluene (1:1) gave unreacted ketone **8a** (8 mg, 15%). Further elution with toluene gave the alcohol **4a** (38 mg, 69%, 81% based on recovered **8a**): ^{1}H NMR (400 MHz, $CDCl_3/CS_2$, 1:2) δ (ppm) 1.19 (d, J = 6.7 Hz, 3H, CH_3), 1.39 (d, J = 6.5 Hz, 3H, CH_3), 1.41 (d, J = 6.3 Hz, 3H, CH_3), 1.72 (d, J = 6.9 Hz, 3H, CH_3), 2.04 (br s, 1H, O*H*), 2.63 (ddd, J = 14.6, 6.3, 5.6 Hz, 1H, H_e), 3.13 (ddd, J = 14.6, 11.8, 10.6 Hz, 1H, H_d), 3.20 (d of sept, J = 6.7, 2.4 Hz, 1H, H_g), 3.25 (d of sept, J = 6.5, 5.7 Hz, 1H, H_a), 3.36 (dd, J = 4.6, 5.7 Hz, 1H, H_b), 3.71 (ddd, J = 11.8, 5.6, 2.4 Hz, 1H, H_f), 5.73 (ddd, J = 10.6, 6.3, 4.6 Hz, 1H, H_c); ^{13}C NMR (125.7 MHz, C_6D_6/CS_2, 1:2) δ (ppm) 19.4, 21.5, 24.8, 28.7, 28.8, 31.2, 33.2, 49.1, 58.1, 68.3, 69.4, 70.0, 135.1, 135.3 (2C), 135.4, 138.8, 138.9, 139.7, 140.1, 141.4, 141.5, 141.6, 141.74, 141.77, 141.8, 141.9, 142.1, 142.2, 142.6, 142.7, 143.2, 144.5, 144.59, 144.66, 144.7, 145.0, 145.1, 145.32, 145.37, 145.4, 145.42, 145.48, 145.78, 145.91, 146.0, 146.1, 146.2, 146.31, 146.36, 146.4, 147.0, 147.4, 147.5, 155.9, 156.5, 157.6, 157.8; HR-MS (FAB) calcd for $C_{70}H_{20}O$ (M^+) 876.1514, found 876.1523.

Alcohol 5, [3′-Phenyl-3′-(α-hydroxybenzyl)-1,2-cyclopropano]buckminsterfullerene. Compound **5** was obtained by DIBAL-H reduction of ketone **9** which was prepared by the method of Bingel.[16] To a solution of ketone **9** (52 mg, 5.7 × 10^{-5} mol) in 30 mL of dry toluene was added DIBAL-H (87 μL

of 1 M solution in toluene) with stirring. The reaction was performed in a foil-wrapped vessel, under argon. After 3.5 h, an additional 40 mL of toluene and 240 μL of DIBAL-H were added. After 2.5 h an additional 580 μL of 1 M DIBAL-H was added. After 24 h, TLC showed quantitative conversion to a lower R_f product (R_f = 0.65, toluene, SiO_2). The reaction mixture was extracted with water, dried with $MgSO_4$, filtered, and evaporated to dryness in vacuo, yielding 45 mg of crude product (87%). This material was purified by chromatography on silica gel with toluene/hexanes (3:1), affording 6.5 mg of analytically pure product: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 2.67 (d, 1 H, J = 5.0 Hz), 6.44 (d, 1 H, J = 5.0 Hz), 7.5 (m, 10 H); ^{13}C NMR (75.0 MHz, $CDCl_3$) d (ppm) 4 resonances < 100 ppm at 57.49, 72.35, 78.42, 79.10 corresponding to the 4 nonaromatic carbons in the structure, a cluster of 56 resolvable peaks was observed at ~122−151 ppm corresponding to most of the peaks expected from the 70 aromatic carbons in the molecule; MS (LSIMS) 915.9 (expected 916.1).

Enzyme Assays. Compounds were assayed at 25 °C using recombinant HIV protease (mutant Q7K).[17] Assays were performed in 50-μL volume under final conditions of 50 mM NaOAc, pH 5.5, 1.0 M NaCl, 5% glycerol, 1% organic solvent (inhibitor dissolved in either NMP or DMSO as noted in text), 2 mM EDTA. The inhibitor was preincubated with the enzyme for 5 min at which time the complexation was initiated by addition of substrate. The complexation was quenched at <15% product formation by the addition of 10 μL of 10% TFA. The cleavage products of the substrate peptide H-Lys-Ala-Arg-Val-Tyr-*p*-nitro-Phe-Glu-Ala-Nle-NH_2 (Bachem) were assayed by HPLC using a 10−40% (acetonitrile, 0.1% TFA)/(water, 0.1% TFA) gradient over 30 min at 1 mL/min. The product was quantitated by integration of peak areas followed by comparison to product standard curves. Determination of kinetic constants was done with the program KinetAsyst (IntelliKinetics). No special precautions were taken during the binding experiments to avoid ambient light and/or eliminate oxygen from the reaction media. However, a control assay using argon-sparged buffers and inhibitor solutions, as well as foil-wrapped assay tubes and subdued lighting, showed full inhibition by compound **5**.

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Supporting Information Available: 2D T-ROESY NMR spectrum of *cis,trans*-alcohol **4a,** solution-determined structure of compound **8a**, and space-filling representation of the docked structure of *all-cis*-alcohol **4b** (3 pages). Ordering information can be found on any current masthead page.

References

(1) (a) Debouck, C. The HIV-1 Protease as a Therapeutic Target for AIDS. *AIDS Res. Human Retrovir.* **1992**, *8*, 153−164. (b) Hirsch, M. S.; D'Aquila, R. T. Therapy for Human Immunodeficiency Virus Infection. N. *Engl. J. Med.* **1993**, *328*, 1686−1695.

(2) Friedman, S. H.; DeCamp, D. L.; Sijbesma, R. P.; Srdanov, G.; Wudl, F.; Kenyon, G. L. Inhibition of the HIV-1 Protease by Fullerene Derivatives - Model Building Studies and Experimental Verification. *J. Am. Chem. Soc.* **1993**, *115*, 6506−6509.

(3) For related work in this area, see: (a) Nakamura, E.; Tokuyama, H.; Yamago, S.; Shiraki, T.; Sugiura, Y. Biological Activity of Water-Soluble Fullerenes. Structural Dependence of DNA Cleavage, Cytotoxicity, and Enzyme Inhibitory Activities Including HIV-Protease Inhibition. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 2143−2151. (b) Schuster, D. I.; Wilson, S. R.; Schinazi, R. F. Anti-Human Immunodeficiency Virus Activity and Cytotoxicity of Derivatized Buckminsterfullerenes. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1253−1256. (c) Jensen, A. W.; Wilson, S. R.; Schuster, D. I. Biological Applications of Fullerenes. *Bioorg. Med. Chem.* **1996**, *4*, 767−779. (d) Schinazi, R. F.; Sijbesma, R.; Srdanov, G.; Hill, C. L.; Wudl, F. Synthesis and Virucidal Activity of a Water-Soluble, Configurationally Stable, Derivatized C_{60} Fullerene. *Antimicrob. Agents Chemother.* **1993**, *37*, 1707−1710.

(4) Wlodawer, A.; Miller, M.; Jaskolski, M.; Sathyanarayana, B. K.; Baldwin, E.; Weber, I. T.; Selk, L. M.; Clawson, L.; Schneider, J.; Kent, S. B. X-Ray Structure of the Empty HIV-1 Protease. *Science* **1989**, *245*, 616−621.

(5) Meng, E. C.; Shoichet, B. K.; Kuntz, I. D. Automated Docking With Grid-Based Energy Evaluation. *J. Comput. Chem.* **1992**, *13*, 505−524.

(6) Molecular Modeling System SYBYL, Version 6.2; Tripos Associates, Inc.

(7) Gschwend, D. A.; Kuntz, I. D. Orientational Sampling and Rigid-Body Minimization in Molecular Docking Revisited − On-The-Fly Optimization and Degeneracy Removal. *J. Comp. Mol. Des.* **1996**, *10*, 123−132.

(8) Michael Connolly, University of California, San Francisco.

(9) Ganapathi, P. S.; Friedman, S. H.; Kenyon, G. L.; Rubin, Y. Sequential "Double-Michael" Additions of Dienolates with C_{60}: Rapid Access to Sterically Congested Buckminsterfullerene Derivatives with Defined Stereochemistry. *J. Org. Chem.* **1995**, *60*, 2954−2955.

(10) By addition of two C_{2v}-symmetric groups, such as the carbenoid leading to **3**, at junctions between two six-membered rings of C_{60}, up to eight regioisomers can result. By decreasing the symmetry of the added group, the number of possible isomers is greatly multiplied because of the inherent stereoisomerism in the corresponding products.

(11) Franz, A.; An, Y.-Z.; Ganapathi, P. S.; Neier, R.; Rubin, Y. Tandem Cycloadditions of *N*,*O*-Ketene *N*-1,3-Butadienyl-*N*-alkyl-*O*-silylacetals with C_{60}: A Straightforward Stereoselective Synthesis of Bicyclic Derivatives of 1,2,3,4-Tetrahydrobuckminsterfullerene. In *Fullerenes Proceedings Series: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials*; Kadish, K. M., Ruoff, R. S., Eds.; 1996; Vol. 3, pp 1326−1341.

(12) Czarnik, A. W.; Ellman, J. A. Special Issue on Combinatorial Chemistry. *Acc. Chem. Res.* **1996**, *29*, 112−170.

(13) High-pressure Diels−Alder reactions will be attempted shortly.

(14) Jung, M. E. In *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Eds.; Pergamon Press: New York, 1991; Vol. 4, pp 1−67.

(15) Hwang, T.-S.; Shaka, A. J. Cross Relaxation without TOCSY: Transverse Rotating-Frame Overhauser Effect Spectroscopy (T-ROESY). *J. Am. Chem. Soc.* **1992**, *114*, 3157−3159.

(16) Bingel, C. Cyclopropylation of Fullerenes. *Chem. Ber.* **1993**, *126*, 1957−1959.

(17) Rose, J. R.; Salto, R.; Craik, C. S. Regulation of Autoproteolysis of the HIV-1 and HIV-2 Proteases with Engineered Amino Acid Substitutions. *J. Biol. Chem.* **1993**, *268*, 11939−11945.

(18) (a) Yamago, S.; Tokuyama, H.; Nakamura, E.; Kikuchi, K.; Kananishi, S.; Sueki, K.; Nakahara, H.; Enomoto, S.; Ambe, F. *In vivo* Biological Behavior of a Water-Miscible Fullerene: ^{14}C Labeling Absorption, Distribution, Excretion and Acute Toxicity. *Chem. Biol.* **1995**, *2*, 385−389. (b) Bullard-Dillard, R.; Creek, K. E.; Scrivens, W. A.; Tour, J. M. Tissue Sites of Uptake of ^{14}C-Labeled C_{60}. *Biiorg. Chem.* **1996**, *24*, 376−385. (c) Moussa, F.; Trivin, F.; Céolin, R.; Hadchouel, M.; Sizaret, P. Y.; Greugny, V.; Fabre, C.; Rassat, A.; Szwarc, H. Early Effects of C_{60} Administration in Swiss Mice: A Preliminary Account for *in vivo* C_{60} Toxicity. *Fullerene Sci. Technol.* **1996**, *4*, 21−29.

(19) For recent work on combinatorial libraries on molecular scaffolds, see: (a) Still, W. C. Discovery of Sequence-Selective Peptide Binding by Synthetic Receptors Using Encoded Combinatorial Libraries. *Acc. Chem. Res.* **1996**, *29*, 155−163. (b) Carell, T.; Wintner, E. A.; Sutherland, A. J.; Rebek, J.; Dunayevskiy, Y. M.; Vouros, P. New Promise in Combinatorial Chemistry: Synthesis, Characterization, and Screening of Small-Molecule Libraries in Solution. *Chem. Biol.* **1995**, *2*, 171−183.

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