S-1153 (AG1549) is perhaps the most promising non-nucleoside inhibitor of HIV-1 reverse transcriptase currently under development as a potential anti-AIDS drug, because it has a favorable profile of resilience to many drug resistance mutations. We have determined the crystal structure of S-1153 in a complex with HIV-1 reverse transcriptase. The complex possesses some novel features, including an extensive network of hydrogen bonds involving the main chain of residues 101, 103, and 236 of the p66 reverse transcriptase subunit. Such interactions are unlikely to be disrupted by side chain mutations. The reverse transcriptase/S-1153 complex suggests different ways in which resilience to mutations in the non-nucleoside inhibitors of reverse transcriptase binding site can be achieved.

The introduction of highly active antiretroviral therapy involving the use of multidrug combinations has resulted in dramatic falls in death rates from HIV infection and AIDS for patients receiving such treatment (1, 2). However, because of the high replication rate of HIV (3), which allows a rapid selection of escape mutants, these current drug regimens are likely to become increasingly ineffective with time. To be able to effectively treat HIV infection in the future, further new drugs with activity against the emerging drug-resistant viruses will be required. The non-nucleoside inhibitors of reverse transcriptase (NNRTIs) are now established as part of multidrug combinations for treating HIV infection (4, 5). So-called "first generation" NNRTIs, such as nevirapine and delavirdine (U-90152), are generally very susceptible to the effects of single point resistance mutations within RT (6, 7). In contrast, more recent "second generation" NNRTIs, such as efavirenz (DMP-266) (8), the carboxanilide, UC-781 (9), and certain quinoxalines (10), demonstrate much greater resilience to the presence of such mutations within RT.

Previous crystallographic studies have shown a common binding site for many classes of NNRTI, with a high degree of overlap for chemically divergent compounds (11–18). Crystallographic and kinetic studies indicate the mechanism of inhibition of NNRTIs is via a distortion of the catalytic aspartate residues in the polymerase active site (19, 20). More recently structural studies have revealed some common factors that contribute to the second generation properties of UC-781 (21). Such factors include a combination of a nonaromatic moiety of optimal size that contacts the aromatic residues Tyr-181, Tyr-188, and Trp-229 at the top of the NNRTI pocket. Additionally, the presence of main chain hydrogen bonding and an overall small compound bulk, thereby allowing rearrangement within a mutated drug binding site, appear to be important. A new NNRTI S-1153 (also now referred to as AG1549) has recently been described (Scheme 1), which appears to have better resilience properties than any previously reported compound (22).

S-1153 is also structurally distinct from other second generation inhibitors such as efavirenz (8) and UC-781 (9) (Scheme 1).

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S-1153 retains full activity against the clinically most common NNRTI resistance mutation, Lys-103 → Asn, compared with the 47-fold loss of activity seen for nevirapine (Table I). Almost full activity is also retained for mutations at codons 106, 188, 190, and 227, whereas nevirapine shows >74-fold weaker binding to these mutants (22). High level resistance to S-1153 requires at least two mutations (e.g. Val-106 → Ala and Phe-227 → Leu, or Lys-103 → Thr, Val-106 → Ala and Leu-234 →
Ile (22). Indeed even some double mutations have only a limited effect, thus Val-106 → Ala and Tyr-181 → Cys result in only an 8-fold loss of activity (Table I).

To investigate the structural basis of this remarkable resilience, we have determined a crystal structure of S-1153 in complex with its molecular receptor HIV-1 RT. Knowledge derived from this study should be of value in the design of novel inhibitors that are active against drug-resistant HIV strains. Further, such drugs will be of importance for the continued effective treatment of HIV infection and AIDS.

MATERIALS AND METHODS

Crystalization and Data Collection—Crystals of the complex of HIV-1 RT with S-1153 (synthesized at Shionogi and Co., Ltd.) were grown and treated prior to data collection as described previously (23). X-ray data were collected at the Synchrotron Radiation Source (Daresbury, UK) on station PX9.6 using an ADSC Quantum-4 CCD detector. A crystal was initially frozen in liquid propane and then maintained at 100 K in a stream of nitrogen gas during data collection. Indexing and integration of data images were carried out with DENZO, and data were merged with SCALEPACK (24). The unit cell dimensions are closest to our F crystal form (25). Details of the x-ray data statistics are given in Table II. The data are reasonably complete and reliable to 2.5 Å resolution.

Structure Determination and Refinement—The orientation and position of HIV-1 RT in the unit cell were determined using rigid body refinement with XPLOR (28), using the RT9-CI-TIBO complex (1rev) (131) as the initial model. The structure was first refined with XPLOR (26) and then with CNS (27), using positional, simulated annealing and individual B-factor refinement with bulk solvent correction and anisotropic B-factor scaling. Model rebuilding was carried out on an Evans and Sutherland ESV workstation using FRODO (28).

The R factor for the final model of RT8-1153 was 0.242, for all data in the range of 30.0–2.5 Å resolution with r.m.s. deviations from ideality of 0.0095 Å and 1.54° for bond lengths and bond angles, respectively. Table II shows the statistics on the refinement and quality of the structure. All figures were produced using BOBSCRIPT (29), a modified version of MOLSCRIPT (30), and rendered with RASTER3D (31).

RESULTS

S-1153 Bound Conformation: Comparison with Other NNRTIs—An “omit” electron-density map for S-1153 unambiguously revealed the orientation and conformation of the inhibitor in the NNRTI binding site (Fig. 1). A comparison of the bound conformation of S-1153 with the first generation NNRTI, nevirapine, is shown in Fig. 2a, whereas an overlay of S-1153 with the second generation NNRTI, UC-781, is shown in Fig. 2b. There is a partial overlap between the cyclopropyl group of nevirapine, part of the furanyl ring of UC-781, and the isopropyl group of S-1153. These structural features position the side chain of Tyr-181 so as to give tight binding (15). For S-1153, the 3,5-dichlorophenyl ring is situated further toward the “top” of the NNRTI pocket than the equivalent aromatic ring for nevirapine, TNK-651 (15), or GCA-186 (32). This reflects the close association of the 3,5-dichlorophenyl ring with the side chain of Trp-229, which appears to be repositioned higher in the pocket because of a conformational change in the 9β, 10β-diphenylstrands, caused by the interaction between the pyridyl and carbamate groups of S-1153 with Pro-236 in the 10β-p11 loop (Fig. 2a). As a result, the 9β, 10β-diphenylstrands are pushed away from the pocket giving a more open pocket entrance (16) and allowing the side chain of Trp-229 to sit higher. The magnitude of this conformational change is exemplified by the mean displacement of α-carbon positions for residues 227–241 in different RT/NNRTI complexes. For RT/nevirapine and RT/UC-781 this is 1.3 Å, whereas for RT/S-1153 and RT-nevirapine this displacement is 3.2 Å, and for RT/S-1153 and RT/UC-781 it is 3.6 Å.

Comparison of sensitivities of HIV-1 containing mutations within the reverse transcriptase to NNRTIs S-1153 and nevirapine

| HIV-1 mutant | $EC_{50}$ (mutant)/$EC_{50}$ (wild type) |
|--------------|-----------------------------------------|
| Leu-106 → Ile | 3.0/2.1 |
| Lys-103 → Asn | 1.0/44.1 |
| Val-106 → Ala | 4.5/>>74 |
| Tyr-181 → Cys | 13.5/>>74 |
| Tyr-185 → Cys | 1.5/>>74 |
| Gly-190 → Ala | 1.1/>>74 |
| Phe-227 → Leu | 1.5/4.1 |
| Leu-234 → Ile | 21.9/0.5 |
| Pro-236 → Leu | 3.5/3.1 |
| Val-106 → Ala & Tyr-181 → Cys | 8.1/>>74 |

$EC_{50}$ is defined as the concentration of compound required to inhibit syncytia formation in HIV-infected cells (adapted from Ref. 22).

TABLE I

Statistics for crystallographic structure determinations

| Data collection details | PX9.6 SRS |
|-------------------------|----------|
| Wavelength (Å) | 0.87 |
| Collimation (mm) | 0.2 × 0.2 |
| Unit cell (a,b,c in Å) | 135.7, 118.0, 67.3 |
| Resolution range (Å) | 30.0–2.5 |
| Observations | 10,8379 |
| Unique reflections | 35,008 |
| Completeness (%) | 90.9 |
| Refinements with $R_{merge}/R_{free}$ | 29,394 |
| $R_{merge}/R_{free}$ (%) | 2.5 |
| Outer resolution shell | 2.5–2.5 |
| Unique reflections | 3261 |
| Completeness (%) | 96.2 |
| Refinements with $R_{merge}/R_{free}$ | 1859 |

TABLE II

Fig. 1. Simulated annealing omit electron density map showing the bound S-1153 in the NNRTI pocket of HIV-1 RT. The map is contoured at 3σ.
Å. The carbamoyloxymethyl group of S-1153 has little overlap with groups in other RT/NNRTIs complexes except for delavirdine, where the carbonyl oxygen is in a similar position (16).

**Hydrophobic Interactions of S-1153 with RT**—The interactions of S-1153 with HIV-1 RT are shown in Figs. 3 and 4. As with all other NNRTIs there are a wide range of hydrophobic contacts between inhibitor and protein. The 3,5-dichlorophenyl group is positioned edge on to the plane of the indole ring of Trp-229, making extensive favorable contacts with a closest approach of 3.7 Å. There are also contacts between the 3,5-dichlorophenyl group and the aromatic rings of Tyr-181 and Tyr-188 (the latter also contacts the sulfur atom of the inhibitor). The other substituents of the imidazole ring make many interactions with the protein. The pyridyl group contacts the side chain of Phe-227 and the main chain of His-235; this group is positioned parallel with the side chain of Pro-236 but approaches no closer than 4.5 Å. The isopropyl group contacts the main chain of Tyr-188 and in addition the side chain of Val-106. The methyl carbamoyloxymethyl group contacts a number of residues at the base of the pocket including Lys-101, Lys-102, and Lys-103 (Fig. 3.).

**Main Chain Hydrogen Bond Interactions of S-1153 with RT**—The most surprising feature of the interaction of S-1153 with the NNRTI binding site is the series of three hydrogen bonds with the protein main chain of RT (Figs. 3 and 4). The carbamoyloxymethyl group of S-1153 forms two direct hydrogen bonds with the protein main chain: from its nitrogen to the main chain carbonyl of Pro-236 and from the terminal oxygen to the main chain nitrogen of Lys-103. The conformational rearrangement of the β9-β10-β11 strands described above results in Pro-236 shifting by about 3.5 Å, breaking a main chain hydrogen bond from its carbonyl to Lys-103, and positioning it instead to hydrogen bond to the carbamate nitrogen of S-1153. A final hydrogen bond is mediated via a water molecule from an imidazole nitrogen of S-1153 to the main chain carbonyl of Lys-101 (Fig. 4).

**DISCUSSION**

Given the substantial data base of structures of RT/NNRTI complexes, we are in a position to dissect out structural features responsible for the resilience of S-1153 to drug resistance mutations within RT. The most remarkable feature of the interaction of S-1153 with HIV RT is the network of three hydrogen-bonding interactions with the main chain, two of which are direct to the protein, whereas one is mediated by a water molecule. The presence of an appropriately positioned carbamate group provides two of these hydrogen bonds (to residues 103 and 236). This is entirely unprecedented for an
RT/NNRTI complex. Some inhibitors for example, nevirapine, α-APA (12), and BM+21.1326(17) bind to RT without any main chain hydrogen bonding. There are several examples of NNRTIs with single hydrogen-bonding interactions with the main chain. Thus in the case of CI-TIBO, emivirine (MKC-442), TNK-651, UC-10, UC-38, UC-84, UC-781, each inhibitor has an interaction with the main chain carbonyl of Lys-101 (13, 15, 21). Delavirdine (U-90152) has a pair of hydrogen bonds from its carbonyl oxygen and indole nitrogen groups to the main chain of Lys-103 (16). Hydrogen bonding to the main chain of Pro-236 as observed here for S-1153 has not been previously reported for any other NNRTI. The rearrangement of the β9-β10-β11 sheet reported here, which is necessary for the Pro-236 main chain hydrogen bonding interaction with S-1153, is facilitated by the great flexibility of this part of the NNRTI pocket that we have previously characterized through the substantial differences in conformation observed for complexes with TNK-651 (15) and delavirdine (16). Such flexibility presents addi-
tional opportunities for favorable interactions but also makes the job of predicting inhibitor interactions with RT from modeling studies problematic.

It seems likely that the extensive network of main chain inhibitor hydrogen bonds observed contribute substantially to the free energy of binding for the inhibitor. In this case we would expect them to confer favorable resilience properties to mutation within the protein, because side chain changes will generally have no direct effect on such interactions. Indeed, tissue culture experiments show that for HIV to escape S-1153, multiple mutations in RT are required (22). Examples of this generally have no direct effect on such interactions. Indeed, mutation within the protein, because side chain changes will enable studies problematic.

But also makes the small nonaromatic moieties of other second generation NNRTIs (7). We have suggested that this may be in common with the double mutant and this change presumably acts directly. Model building studies indicate that the Leu-234 to ile mutation gives unfavorable contacts with the S-1153 pyridyl group as a result of the close approach of the CG2 methyl moiety of the isoleucine side chain. The mutation at 103 is, however, more intriguing; a change at codon 103 from lysine to asparagine is the most commonly observed NNRTI in all NNRTIs (19). S-1153 pyridyl group as a result of the close approach of the CG2 methyl moiety of the isoleucine side chain. The mutation at 103 is, however, more intriguing; a change at codon 103 from lysine to asparagine is the most commonly observed NNRTI in all NNRTIs (19). S-1153 pyridyl group as a result of the close approach of the CG2 methyl moiety of the isoleucine side chain. The mutation at 103 is, however, more intriguing; a change at codon 103 from lysine to asparagine is the most commonly observed NNRTI in all NNRTIs (19). S-1153 pyridyl group as a result of the close approach of the CG2 methyl moiety of the isoleucine side chain. The mutation at 103 is, however, more intriguing; a change at codon 103 from lysine to asparagine is the most commonly observed NNRTI in all NNRTIs (19). S-1153 pyridyl group as a result of the close approach of the CG2 methyl moiety of the isoleucine side chain.

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S-1153 Bound to HIV-1 Reverse Transcriptase

Second generation NNRTIs can employ alternative strategies to minimize the effects of many drug resistance mutations within RT. S-1153 has distinctive features, when compared with second generation compounds such as UC-781, in its interactions with RT. This leads to the hope that a range of further NNRTIs can be designed to progressively counter newly emerging resistant HIV strains resulting from the strong selective pressure of effective chemotherapeutics.

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Binding of the Second Generation Non-nucleoside Inhibitor S-1153 to HIV-1 Reverse Transcriptase Involves Extensive Main Chain Hydrogen Bonding
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