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Perspective

New Approaches toward Anti-HIV Chemotherapy[‡]

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Introduction

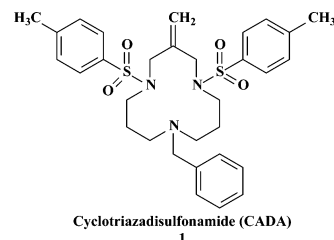
The development of new antiretroviral drugs is a dynamic process that is continuously fueled, at the basic level, by the identification of new molecular targets for chemotherapeutic intervention and at the clinical level by the problems (i.e., adherence, tolerability, toxicity, virus-drug resistance) encountered with existing drugs. En route from their discovery as new anti-HIV entities to their eventual use for the treatment of HIV infection, new antiretroviral drugs have to cross many hurdles, and at each point of the developmental process, emerging pitfalls have to be balanced against potential benefits at the end. This makes it often unpredictable or at least unforeseeable as to which compounds would make it all the way from the beginning till the end, the final approval for clinical use.

Here, I will discuss a number of strategies for the chemotherapy and chemoprophylaxis of HIV infections, based on new compounds that have recently entered the antiretroviral arena. Some of these compounds have just been identified as anti-HIV agents; others have already gone through the whole developmental process to their final approval as antiretroviral drugs. This review on new trends and approaches toward antiretroviral drugs is not meant to be exhaustive but rather exemplary for the different strategies that could be pursued and those that have already proved to be successful. These strategies are focused on the following targets: (i) CD4 as the primary cell receptor for viral entry into the cell; (ii) gp120 as the viral glycoprotein involved in virus adsorption to the cells; (iii) CXCR4 and CCR5 as the corecep-

tors for viral entry; (iv) gp41 as the viral glycoprotein required for virus-cell fusion; substrate (dNTP) binding site of HIV reverse transcriptase as the point of interaction for the (v) NRTIs (“nucleoside reverse transcriptase inhibitors”) and (vi) NtRTIs (“nucleotide reverse transcriptase inhibitors”); (vii) nonsubstrate binding site of the HIV-1 reverse transcriptase as the point of interaction for the NNRTIs (“non-nucleoside reverse transcriptase inhibitors”); (viii) integration of the proviral DNA into the host cell genome by the HIV integrase; (ix) proviral DNA expression (transcription, transactivation); and (x) HIV protease as the target for both peptidomimetic and nonpeptidic inhibitors of the HIV protease. For a comprehensive review on new anti-HIV agents in preclinical or clinical development, see ref 1.

Cellular CD4 Receptor Down-Modulators

The CD4 cell surface molecule serves as the primary receptor for HIV infection of its target cells, and thus, drugs that target the CD4 receptor, thereby inhibiting viral entry into the cells, may be considered an attractive approach toward prevention of HIV infection. Cyclotriazadisulfonamide (CADA) (1) was recently shown

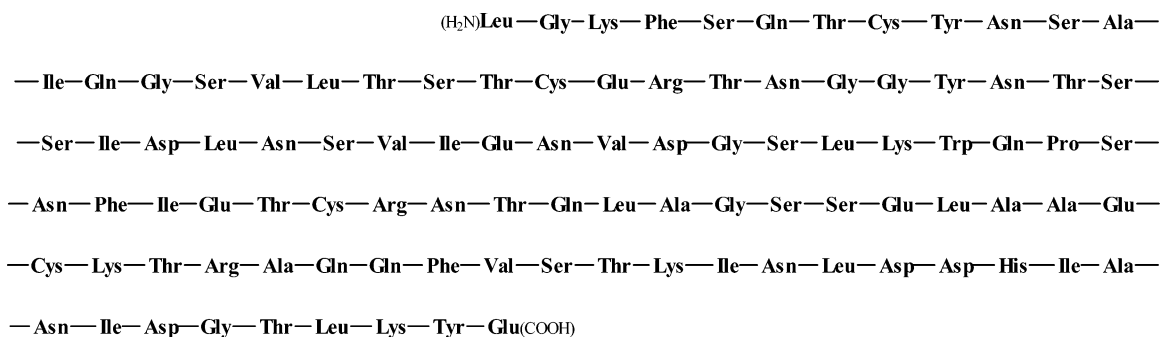


to inhibit HIV (as well as human herpesvirus type 7) infection by down-modulation of the cellular CD4 receptor.² The antiviral activity of a large series of varying

[‡] This paper is dedicated to the memory of Dr. Paul Janssen, outstanding mentor, humanist, and medicinal chemist.

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Chart 1



Cyanovirin-N

2

CADA derivatives correlated closely with their ability to down-modulate CD4 receptor expression.³ CADA is specific for the CD4 receptor; it was not found to alter the expression of any other cellular receptor (i.e., CXCR4, CCR5). Time course experiments revealed that CADA in its mechanism of action differs from that of aurointricarboxylic acid, which binds directly to CD4 and phorbol myristate acetate, which activates protein kinase C²; CADA is assumed to down-regulate CD4 expression at the (post)translational level.³ Further pertaining to the promising character of CADA as an anti-HIV agent was the observation that it proved synergistic in its anti-HIV activity when combined with NRTIs (i.e., zidovudine, lamivudine, zalcitabine, abacavir), NtRTIs (i.e., tenofovir), NNRTIs (i.e., nevirapine, delavirdine), PIs (protease inhibitors lopinavir, saquinavir, indinavir, nelfinavir, amprenavir, and ritonavir), as well as the gp41 fusion inhibitor T-20 (enfuvirtide), the CXCR4 antagonist AMD3100, and the mannose-specific plant lectins from *Galanthus nivalis* (GNA) and *Hippeastrum* hybrid (HHA).⁴

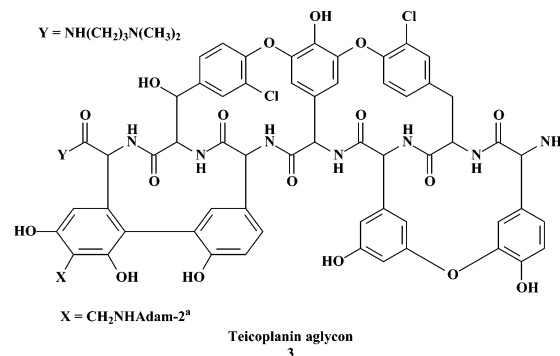
Virus Attachment Inhibitors

The above-mentioned plant lectins derived from GNA (Snowdrop) and HHA (Amaryllis) were shown to interrupt the viral entry process by interfering with the viral envelope glycoprotein gp120.⁵ In fact, when HIV-1 was made resistant to GNA and HHA following repeated passages in the presence of the compounds, several amino acid changes were noted in gp120 but not gp41; the vast majority of these amino acid changes occurred at the N-glycosylation sites (at the S or T residues), and the degree of resistance to the plant lectins invariably correlated with an increasing number of mutated glycosylation sites.⁶ There was no cross-resistance of plant lectin-resistant viruses to other viral entry inhibitors such as dextran sulfate, bicyclams (i.e., AMD3100), chicoric acid, or T-20 (enfuvirtide). The plant lectins represent a unique class of anti-HIV agents with an entirely novel HIV drug resistance profile;⁶ they are endowed with a number of interesting properties (i.e., availability, formulation, stability, efficacy, safety) that make them primary candidate drugs to be considered for potential topical use as microbicides for the prevention of the sexual transmission of HIV infection.⁵

Equally well qualifying as a potential microbicide to prevent the transmission of HIV and AIDS is cyanovirin-N (**2**, Chart 1), a 11 kDa protein originally isolated from the cyanobacterium *Nostoc ellipsosporum*.⁷ Cy-

anovirin-N has a uniquely high affinity for gp120: it impairs both CD4-dependent and -independent binding of gp120 to the target cells; it blocks CD4-induced binding of gp120 with CXCR4, and it dissociates bound gp120 from target cells.⁸ Cyanovirin-N exists as either a quasi-symmetric two-domain monomer or a domain-swapped dimer, as demonstrated by nuclear magnetic resonance and crystallography.⁹

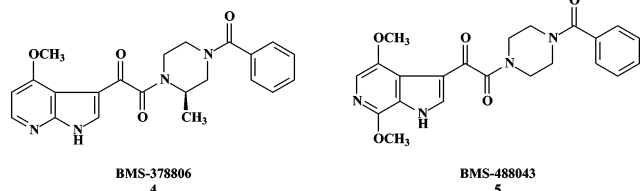
The aglycons of the glycopeptide antibiotics vancomycin, teicoplanin, and eremomycin, in particular those containing substituents of a preferably hydrophobic nature, were found to display activity against HIV-1, HIV-2, and Moloney murine sarcoma virus at the lower micromolar concentration range. Time-of-addition experiments carried out with one of the prototype compounds, namely, the teicoplanin aglycon **3**, have indi-



cated that this type of compound interferes with viral entry, probably at the virus adsorption step, because their anti-HIV activity was lost if added at 1–2 h after infection. The binding site (probably located at gp120) for these compounds still remains to be resolved. Glycopeptide antibiotic aglycon derivatives could be envisaged as potential lead compounds for application as microbicides against sexual HIV transmission.¹⁰

Recently, a new class of HIV-1 attachment inhibitors [prototype: 4-benzoyl-1-[4-methoxy-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl]oxoacetyl]-2-(*R*)-methylpiperazine (BMS-378806) (**4**)] has been identified that interferes with CD4–gp120 interactions.¹¹ Compound **4** binds directly to gp120 at a stoichiometry of approximately 1:1, with a binding affinity similar to that of soluble CD4. The potential target site of **4** was localized to a specific region within the CD4 binding pocket of gp120; compound **4** proved inactive against CD4-independent viral infections, confirming gp120–CD4 interactions as the target

for its anti-HIV activity.¹² The 4-resistant variants have been isolated with amino acid substitutions (M426L and M475I) in the CD4-binding pocket of gp120, again confirming that **4** targets gp120. Compound **4** displays many favorable pharmacological traits such as low protein binding, minimal human serum effect on anti-HIV-1 potency, good oral bioavailability in animal species, and a clean safety profile in initial animal toxicology studies.¹³



Proof of concept that virus attachment inhibitors may be effective in vivo in HIV-1-infected patients has been provided for BMS-488043 (**5**), which is structurally closely related to compound **4**. Like **4**, **5** is assumed to bind to the HIV-1 glycoprotein gp120 and to induce or block conformational changes in the gp120.¹⁴ In HIV-1 infected adults, oral administration of 1800 mg every 12 h for 8 days led after 14 days to a viral load reduction of $>1.0 \log_{10}$ in 67% of the patients, with 42% having a viral load decline of $>1.5 \log_{10}$.¹⁴ The compound was well tolerated, and no serious adverse events were noted.

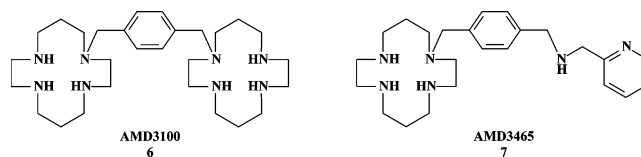
CXCR4 and CCR5 Antagonists

To enter the cells following binding with the CD4 receptor, the HIV particles must interact, again through the viral envelope glycoprotein gp120, with the CXCR4 or CCR5 coreceptor; CXCR4 is the coreceptor for T-lymphotropic (or X4) HIV strains, whereas CCR5 is the coreceptor for macrophage (M)-tropic (or X5) HIV strains. CXCR4 normally functions as the receptor for the chemokine SDF-1 (stromal cell derived factor), and CCR5 does so for RANTES (regulated upon activation, normal T-cell expressed, and secreted) and MIP-1 α and -1 β (macrophage inflammatory proteins), and accordingly, these chemokines inhibit the infectivity of X4 and X5 HIV strains, respectively.

Various low-molecular-weight CXCR4 and CCR5 antagonists have been identified, the prototype of the CXCR4 antagonists being the bicyclam AMD3100 (**6**). Compound **6** is truly specific for CXCR4. It does not interact with any other CXCR or CCR receptor and blocks X4 HIV-1 replication through CXCR4 antagonization.¹⁵ During phase I clinical studies it was found that **6** caused a significant enhancement of the white blood cells (WBC) counts in human volunteers.¹⁶ It was then shown to mobilize hematopoietic stem cells from the bone marrow into the bloodstream, and because this effect proved synergistic with the action of G-CSF (granulocyte-colony stimulating factor), compound **6** is now being pursued (in phase II clinical studies) for stem cell mobilization and transplantation in patients with multiple myeloma or non-Hodgkin lymphoma.

Proof of principle has been provided that CXCR4 antagonists such as **6** are able to suppress in vivo replication of X4 or dualtropic X4/R5 HIV strains.¹⁷ Notably, in HIV-infected individuals with dual (X4/R5) or mixed (X4 + R5) virus at baseline, **6** at a dose as low

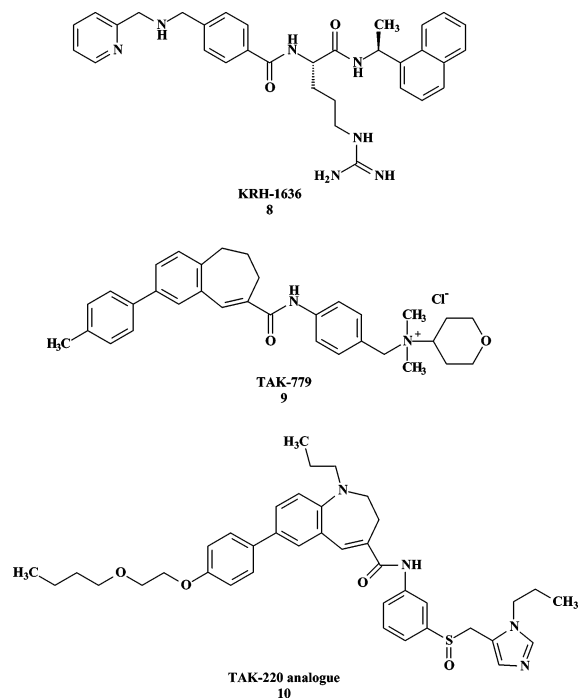
as $5 \mu\text{g kg}^{-1} \text{h}^{-1}$ (continuous infusion) effected a complete loss of X4 virus after 10 days of treatment.¹⁷ An orally bioavailable derivative of AMD3100, namely, AMD070, has recently been described that is as potent a CXCR4 antagonist and anti-HIV agent as AMD3100, and that will be further pursued as a candidate anti-HIV drug in clinical trials.^{18,19}



The structure of AMD070 has yet to be disclosed. Structure-activity relationship (SAR) studies with bicyclam analogues have revealed that the bis-macrocylic structure is not an absolute prerequisite for anti-HIV activity; i.e., AMD3465 (**7**), an AMD3100 derivative in which one of the cyclam rings is replaced by a pyridylmethylene amine moiety, proved to be as potent, if not more so, as AMD3100 as both a CXCR4 antagonist and inhibitor of X4 HIV strains.²⁰

Also, KRH-1636 (**8**), another CXCR4 antagonist, seems to be endowed with an anti-HIV activity profile similar to that of AMD3100. Additionally, compound **8** appears to be duodenally absorbable, at least in rats,²¹ which means that, like AMD070, it may be orally bioavailable.

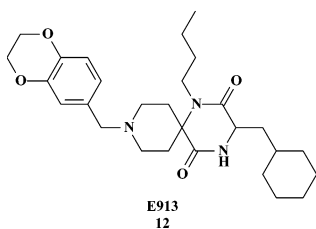
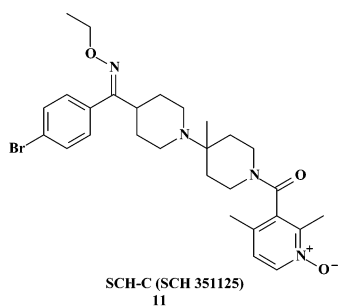
Of the CCR5 antagonists, the quaternary ammonium derivative TAK-779 (**9**) was the first nonpeptidic molecule shown to block the replication of the M-tropic R5 HIV-1 strains (in the nanomolar concentration range) by interaction with CCR5.²² The binding site for **9** has been identified within the transmembrane (TM) helices 1, 2, 3, and 7 of CCR5.²³ Compound **9** is not orally bioavailable and provokes irritation at the injection site. Further research has led to the identification of TAK-220, an orally bioavailable CCR5 antagonist with potent anti-R5 HIV-1 activity.²⁴



TAK-220 was found to inhibit the replication of R5 HIV-1 strains with an EC_{50} of 1.1 nM and an EC_{90} of 13 nM. Oral bioavailability of TAK-220 in fasted rats and monkeys was 9.5% and 28.5%, respectively, and the drug was found to concentrate 2-fold in the lymph fluid (in rats), compared with plasma. The structure of TAK-220 has not been disclosed. A structural analogue of TAK-220, taken from a recent patent application,²⁵ is shown (**10**).

SCH 351125 (SCH-C) (**11**) was the first CCR5 antagonist to be advanced to clinical studies. Compound **11** has potent activity *in vitro* against primary HIV-1 isolates using CCR5 as their cell entry coreceptor; it strongly inhibits the replication of R5 HIV-1 in SCID-hu Thy/Liv mice and shows a favorable pharmacokinetic profile (i.e., good oral bioavailability in rats, dogs, and monkeys).²⁶ Preliminary clinical data based on the oral administration of **11** at 25 mg twice daily for 10 days to a number of HIV-1-infected adults off therapy with other antiretrovirals indicated that **11** is able to achieve a 0.5–1.0 \log_{10} reduction in viral load.²⁷ Starting from **11**, various new, orally bioavailable CCR5 antagonists containing symmetrical 2,6-dimethylisonicotinamides and 2,6-dimethylpyrimidine 5-carboxamides were generated that showed equal or even enhanced affinity for the CCR5 receptor compared to the parent compound.^{28,29}

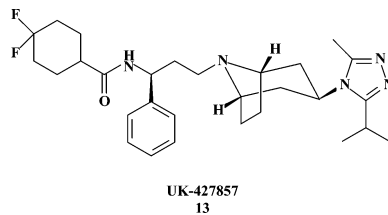
Compound **11** and SCH-350581 (AD101) bind to overlapping but nonidentical sites within a putative ligand-binding cavity formed by the transmembrane (TM) helices 1, 2, 3, and 7. It has been proposed that the binding of these molecules to the TM domain of CCR5 may disrupt the conformation of its extracellular domain, specifically the second extracellular loop, so that it can no longer interact with the crown of the V3 loop of gp120.³⁰



Another class of low-molecular-weight CCR5 antagonists is represented by the spirodiketopiperazine E913 (**12**). Compound **12** specifically blocks the binding of MIP-1 α to CCR5, the MIP-1 α -elicited Ca^{2+} flux, and the replication of both laboratory and primary R5 HIV-1 strains, as well as various multidrug-resistant mono-cyte/M-tropic R5 HIV-1 strains.³¹ From the spirodike-

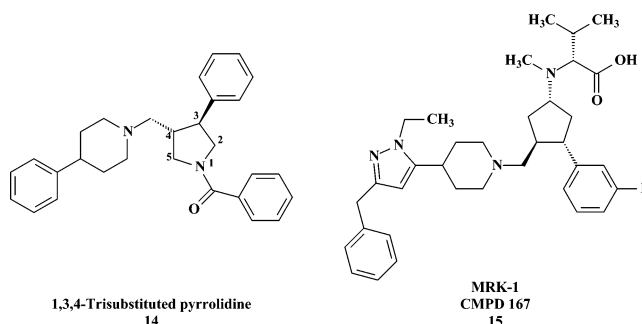
topiperazine class of compounds, **12** was recently quoted as binding only partially to CCR5 while exhibiting much greater anti-HIV activity. This compound was also reported to block R5 HIV-1 replication in HIV-1 (JRFL)-infected hu-PBM NOD-SCID mice and to possess favorable oral bioavailability in rodents.³²

UK-427,857 (**13**), another CCR5 antagonist, has re-



cently been selected as a clinical development candidate drug for the treatment of HIV infection. Compound **13** was reported to display excellent potency against a range of laboratory-adapted and primary isolates utilizing CCR5 for cell entry (at $IC_{90} < 10$ nM) while being inactive against CXCR4-tropic viral isolates.³³ Compound **13** was evaluated in more than 400 volunteers and R5 HIV-1-infected patients where it was well tolerated at doses in excess of those required to block the CCR5 receptor. At a dose of 100 mg twice a day, **13** achieved a mean decrease in viral load of 1.42 \log_{10} from baseline to day 11 [after short-term (10 day) therapy], whereas at a dose of 25 mg once a day, **13** produced a mean viral load reduction of 0.42 \log_{10} .³⁴ Remarkably, the R5 HIV-1 load remained suppressed for at least 10 days after stopping therapy, which suggests that treatment with **13** (and perhaps other CCR5 antagonists as well) may be based on infrequent administration, i.e., once weekly instead of daily.

In addition to the aforementioned CCR5 antagonists, a wealth of primarily 1,3,4-trisubstituted pyrrolidine CCR5 receptor antagonists have been described that possess oral bioavailability and/or potent anti-HIV activity.^{35–39} A representative congener of this 1,3,4-trisubstituted pyrrolidine series is depicted in **14**. The compound's site of interaction with CCR5 has been mapped to a cavity, near the extracellular surface, formed by the TM helices 2, 3, 6, and 7.⁴⁰ This site is overlapping with, yet distinct from, that reported for **9**.²³ Further molecular modeling studies of the binding pocket should help in optimizing the design of new CCR5 antagonists.



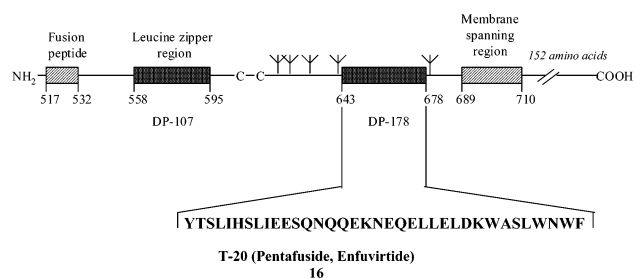
Proof of principle that CCR5 antagonists are efficacious *in vivo* against CCR5-using virus strains has been demonstrated for CMPD 167, designated previously as

MRK-1 (**15**). This compound caused a rapid and substantial (4- to 200-fold) decrease in plasma viremia in rhesus macaques chronically infected with simian immunodeficiency virus (SIV). Also, viral replication could be partially inhibited upon vaginal administration of gel-formulated **15**, pointing to the potential usefulness of this type of compound as a (component of) a topical microbicide to prevent HIV-1 sexual transmission.⁴¹

HIV infection of human lymphoid cells can be inhibited by the membrane-impermeant sulfhydryl blocker 5,5'-dithiobis(2-nitrobenzoic acid), by bacitracin, and by antibodies to protein–disulfide isomerase (PDI).⁴² PDI is a host cell enzyme that cleaves disulfide bonds in the HIV envelope glycoprotein gp120, thus triggering conformational changes required for HIV-cell fusion.^{43,44} On average, two of the nine disulfides of gp120 are reduced during its interaction with the lymphocyte surface after CXCR4 binding prior to fusion.⁴⁵ The PDI-mediated disulfide reduction takes in the gp120/CD4/coreceptor complex and may be the final step that triggers the spring-load action of gp41 (see next section). Whether this PDI-mediated process would be useful as a drug target for chemotherapeutic intervention remains to be explored.

Virus-Cell Fusion Inhibitors

The interaction of the X4 or R5 HIV-1 envelope gp120 with the coreceptors CXCR4 or CCR5, respectively, is followed by a spring-loaded action of gp41 that then anchors through its amino terminus into the target cell membrane and, in doing so, initiates the fusion of the viral envelope with the cellular plasma membrane. At the onset of the fusion process, hydrophobic grooves on the surface of the coiled coil gp41 ectodomain become available for binding with extraneous inhibitors, such as enfuvirtide (T-20, DP-178, pentafuside, Fuzeon) (**16**),



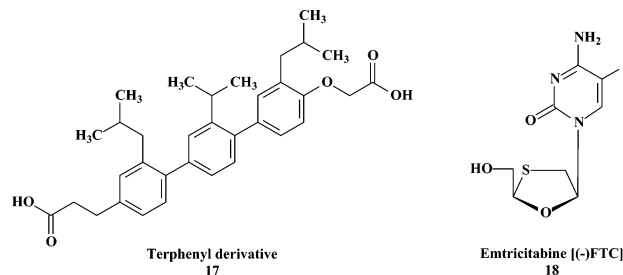
a synthetic, 36-amino-acid peptide corresponding to residues 127–162 of gp41 or residues 643–678 of the gp160 precursor.

In an initial clinical trial carried out with **16** at four doses (3, 10, 30, and 100 mg twice daily, intravenously, for 14 days) in HIV-infected adults, at the highest dose (100 mg, twice daily) **16** achieved a 1.5- to 2.0-fold reduction in plasma HIV RNA by the 15th day, thus providing proof of concept that HIV fusion inhibitors are able to reduce virus replication in vivo.⁴⁶ Meanwhile, two phase III clinical studies, the so-called T-20 versus optimized regimen only studies 1 (TORO 1) and 2 (TORO 2) conducted in North and South America⁴⁷ and Europe and Australia,⁴⁸ respectively, have indicated that enfuvirtide provided a significant antiretroviral benefit [i.e., incremental viral load reduction (0.932 and 0.781 log₁₀ copies per milliliter, respectively)] and an

immunologic benefit [i.e., incremental increases of CD4⁺ cell counts (44 and 275 cells per microliter, respectively)] following a 24-week treatment period. Follow-up studies over 48 weeks have confirmed the 24-week findings, thus supporting the efficacy of enfuvirtide plus optimized background (OB) therapy over OB therapy only.⁴⁹ Further analysis of the virological responses in the TORO 1 and TORO 2 studies has ascertained that the virological responses to enfuvirtide plus OB therapy were directly related to the activity of the background regimen, and improved responses were observed in less advanced and less experienced patients.⁵⁰

Enfuvirtide has to be administered twice daily by subcutaneous injection. This inevitably leads to injection-site reactions including erythema, induration, and nodules, and cysts. Another equally inevitable problem is the production cost for a 5000 Da molecular mass peptide such as enfuvirtide.⁵¹ As for all specific anti-HIV drugs, the emergence of virus-drug resistance should be continuously and carefully monitored. Mutations G36D and V38A within the GIV stretch at positions 36–38 of the amino terminal heptad motif of gp41⁵² and also other mutations seem to emerge promptly in patients upon monotherapy with enfuvirtide.⁵³ Even enfuvirtide-insensitive HIV-1 variants may exist in an enfuvirtide-naïve population, and this may allow virus escape from drug.⁵⁴ However, given the fact that enfuvirtide (as well as SCH-C and RANTES) inhibits the replication of different CCR5-using primary viral isolates in multiple cell types, enfuvirtide (as well as the other viral entry inhibitors) should be further evaluated for its clinical potential as an inhibitor of HIV-1 replication in several settings, including the prevention of maternal–infant transmission and the prevention of sexual transmission by topical application as a microbicide.⁵⁵

Recently, a new strategy targeted at gp41 and aimed at inhibiting virus-cell fusion has been described.^{56,57} This strategy is based on α -helix mimicry. Tris-functionalized 3,2',2''-terphenyl derivatives (**17**) could serve as effective mimics of the exposed N-helical regions of the transient gp41 intermediate and thus potentially trap this structure prior to the six-helix bundle formation, which is required for virus-cell fusion. Compound **17** was indeed found to inhibit HIV-1 mediated cell-to-cell fusion but only at a relatively high concentration (IC₅₀ \approx 15 μ g/mL).⁵⁷ A similar approach, based on short constrained peptides (i.e., C14 linkmid),⁵⁸ also resulted in inhibition of cell-to-cell fusion (IC₅₀ from C14 linkmid: 35 μ M).



Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

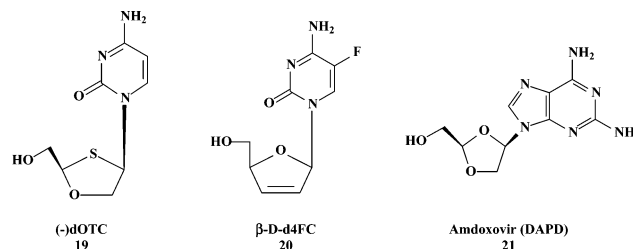
Following zidovudine (AZT, Retrovir), didanosine (ddI, Videx), zalcitabine (ddC, Hivid), stavudine (d4T, Zerit),

lamivudine (3TC, Epivir) (also marketed in combination with zidovudine under the trademark Combivir and with abacavir under the trademark Epzicom), and abacavir (ABC, Ziagen) (also marketed in combination with zidovudine and lamivudine under the trademark Trizivir), emtricitabine (2',3'-dideoxy-3'-thia-5-fluorocytidine, (-)-FTC, previously referred to as Coviracil and now marketed as Emtriva) (**18**) is the seventh 2',3'-dideoxynucleoside analogue that has been officially approved (as of July 2, 2003) for the treatment of HIV infections. Emtricitabine can be conveniently administered as a once-daily dose of 200 mg⁵⁹ for long-term clinical use in HIV-1-infected individuals. A once-daily combination therapy of emtricitabine with didanosine and efavirenz has proven safe and both antivirally and immunologically effective for at least 24 weeks.⁶⁰ In follow-up studies through 60 weeks, once-daily emtricitabine, combined with once-daily didanosine and efavirenz, demonstrated durable and superior virologic efficacy and tolerability, compared to twice-daily stavudine and once-daily didanosine and efavirenz.⁶¹ Emtricitabine has been considered an "ideal drug candidate" because it shows synergism with other antiretrovirals, excellent tolerability, a long intracellular half-life (supporting the once-daily dosing), and, in comparison with lamivudine, 4- to 10-fold higher in vitro potency against HIV.⁶² However, the relative anti-HIV-1 potency of emtricitabine and lamivudine in vitro is dependent on the cell type. In primary cells (peripheral blood mononuclear cells and monocyte-derived macrophages) no significant differences in potency were noted.⁶³

What are the other NRTIs that are under (pre)clinical development and may eventually reach the anti-HIV drug market? These include (\pm)-2'-deoxy-3'-oxa-4'-thiocytidine (dOTC, BCH-10652)⁶⁴ and its 5-fluoro-substituted counterpart FdOTC (Racivir)⁶⁴ and the enantiomeric forms thereof,⁶⁵ as well as β -D-2',3'-dideoxy-2',3'-dideoxy-5-fluorocytidine (β -D-d4FC, RVT, Reverset). The (-)-enantiomer of dOTC (**19**), termed SPD754, shows promising antiviral activity against viruses harboring the M184V and multiple thymidine analogue mutations (TAMs). However, there is a marked reduction in intracellular (-)-dOTC triphosphate concentration in the presence of 3TC, resulting in antagonism of their antiviral activity, which contraindicates the coadministration of SPD754 with 3TC in the clinical setting.⁶⁶

RVT (**20**) corresponds to the 5-fluoro-substituted derivative of β -D-d4C (β -D-2',3'-dideoxy-2',3'-dideoxy-5-fluorocytidine), a compound that was already described in 1986 as a potent and selective anti-HIV agent.⁶⁷ It is noted that β -D-d4FC (RVT) retains activity against HIV isolates harboring mutations in the reverse transcriptase gene that confer resistance to 3TC or AZT;⁶⁸ however, it seems less potent against multi-NRTI-resistant viruses, particularly those carrying the Q151M mutation.⁶⁹ In addition, RVT itself selects for the K65R mutation that confers 5.3- to 8.7-fold resistance to RVT in vitro.⁶⁹ RVT is readily converted to its 5'-triphosphate in human peripheral blood mononuclear cells and interacts synergistically with a number of other anti-HIV agents.⁷⁰ A phase I clinical study has demonstrated that the desired plasma concentrations of RVT can be

easily achieved with an oral dose of 50 mg. RVT may be useful as a once-a-day component for the treatment of NRTI-experienced patients.⁷¹ In the SCID-hu Thy/Liv mouse model, RVT effected a viral load reduction below the detection limit for both the NL4-3 and 3TC-resistant M184V HIV-1 strains.⁷²

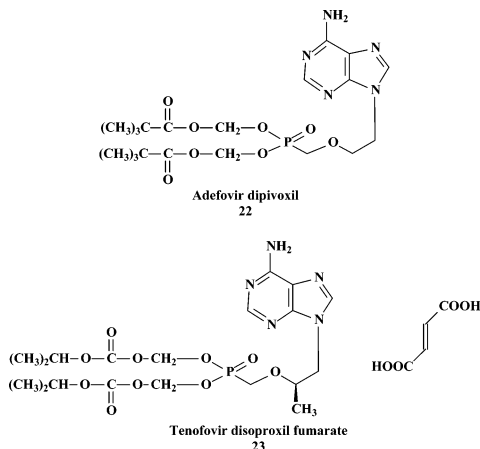


Among the new purine-based 2',3'-dideoxynucleoside analogues, the most advanced is amdoxovir ((-)- β -D-2,6-diaminopurine dioxolane, DAPD, **21**). DAPD is converted by adenosine deaminase to dioxolane guanine (DXG) which is then phosphorylated intracellularly to DXG 5'-triphosphate, the active metabolite.⁷³ DXG 5'-triphosphate (DXG-TP) acts as an alternative substrate/inhibitor of the HIV-1 reverse transcriptase.⁷⁴ Intracellular DXG-TP levels have been demonstrated in peripheral blood mononuclear cells from HIV-infected patients receiving oral DAPD (500 mg twice daily).⁷⁵ DAPD/DXG has proven active against HIV-1 mutants resistant to zidovudine (M41L/D67N/K70R/T215Y/K219Q) and lamivudine (M184V)⁷³ but has decreased activity against viruses carrying the K65R and Q151M mutations.⁷⁶ Also, drug-resistant HIV-1 isolates (including multi-drug-resistant variants with the 69 double codon insertion SS or SG) from patients where standard nucleoside therapy failed remained susceptible to DXG; resistance to DXG was observed only for recombinant isolates harboring the K65R and Q151M double mutation.⁷⁷

Nucleotide Reverse Transcriptase Inhibitors (NtRTIs)

In contrast to the nucleoside reverse transcriptase inhibitors (NRTIs), the nucleotide reverse transcriptase inhibitors (NtRTIs) such as adefovir [9-(2-phosphonyl-methoxyethyl)adenine (PMEA)] and tenofovir [(*R*)-9-(2-phosphonylmethoxypropyl)adenine (PMPA)] are already equipped with a phosphonate group and, therefore, only need two phosphorylation steps to be converted to the active metabolites (PMEApp and PMPApp, respectively). The metabolites then serve as alternative substrates (with respect to the natural substrate dATP) in the reverse transcriptase reaction, where upon their incorporation they act obligatorily as chain terminators.⁷⁸ Akin to the NRTIs such as lamivudine, emtricitabine, and amdoxovir, the NtRTIs adefovir and tenofovir are active not only against HIV but also against hepatitis B virus (HBV), which is not surprising because HBV uses for its replication a reverse transcriptase that is quite similar to that of HIV. Adefovir and tenofovir have been officially approved, in their oral prodrug forms adefovir dipivoxil [bis(pivaloyloxymethyl)-PMEA, Hepsera] (**22**) and tenofovir disoproxil [bis(isopropylloxycarbonyloxymethyl)-PMPA] fumarate (Viread) (**23**),

for the treatment of HBV and HIV infections, respectively.



Tenofovir disoproxil fumarate (TDF) is prescribed as a once-daily dose of 300 mg. Adding TDF (300 mg) to existing antiretroviral therapy for highly treatment-experienced patients with preexisting resistance mutations resulted in significant and durable reductions in HIV-1 RNA levels through week 96. Through 96 weeks of TDF therapy, 48 weeks of which included suboptimal doses of TDF, there was infrequent development of the reverse transcriptase mutation (K65R) associated with TDF resistance (3% of the patients), consistent with the durability of the observed HIV-1 RNA responses.⁷⁹

From an ongoing 3-year randomized, double-blind clinical trial being conducted at 81 sites in the U.S., Europe, and South America, comparing the efficacy and safety of a treatment regimen of TDF, 3TC, and efavirenz to a regimen of d4T, 3TC, and efavirenz in 600 antiretroviral-naïve patients with HIV infections, results from the 48-week data⁸⁰ and subsequently the 96-week data were divulged:⁸¹ both treatment groups showed a mean reduction in HIV RNA load of 3.09 log₁₀ copies per milliliter (baseline: 4.9 log₁₀ copies per milliliter) after 48 weeks; 95% of the patients in the TDF arm compared to 91% in the d4T arm had reductions in HIV RNA load to below 50 copies per milliliter after 96 weeks. Thus, both the d4T and TDF arms showed a similar, high virological response. However, lipid abnormalities (increase in triglyceride and cholesterol levels) were significantly lower in the TDF arm than in the d4T arm. Also, the toxicities (peripheral neuropathy, lipodystrophy, lactic acidosis, pancreatitis) associated with mitochondrial dysfunction through week 96 were markedly lower in the TDF arm than in the d4T arm, while both arms showed a similar renal safety profile. Although this study is still ongoing, the ad interim results point to a similar efficiency but better safety profile of TDF compared to d4T. Whereas at the present time, the combination of TDF + 3TC + efavirenz looks like a most efficient drug regimen, it seems worthwhile to examine in the future a regimen consisting of TDF + (-)-FTC + efavirenz.

In efforts to reduce pill burden and increase convenience of administration, TDF and (-)-FTC have been combined into a single tablet to be taken once daily. The fixed dose combination of TDF (300 mg) and (-)-FTC (200 mg) is bioequivalent to the individual dosage form.

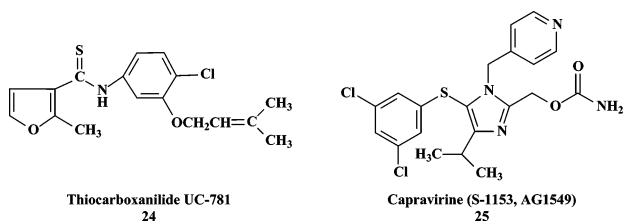
It has been planned to give this fixed dose combination of TDF and (-)-FTC, now marketed under the trademark Truvada, together with efavirenz as two pills and ultimately as one pill once daily. As part of a global access program, TDF and the fixed dose combination of TDF with (-)-FTC are now being made available at no profit for the developing world. It has also been planned to explore the potential of TDF in the pre- and/or postexposure prophylaxis of HIV infection in both women (in Africa and Cambodia) and men (in the U.S.) at high risk for acquiring HIV infection,⁸² and furthermore, it has been planned to use tenofovir as a 1% vaginal (microbicide) gel for the prevention of sexual HIV transmission.⁸²

Although in an animal model for HIV transmission through breast-feeding topical administration of low-dose TDF did not protect infant macaques against multiple oral exposures of simian immunodeficiency virus (SIV),⁸³ it was concluded that systemic administration of potent antiviral compounds such as tenofovir may be the best chemoprophylactic strategy to reduce HIV transmission via breast-feeding. In fact, in a monotherapy trial with TDF in 10 chronically HIV-1-infected antiretroviral-naïve individuals, the drug achieved a 1.5 log₁₀ reduction in HIV-1 RNA levels following a 3-week treatment course, a response that was considered as robust as that observed with ritonavir monotherapy.⁸⁴ It now seems worth examining whether the use of TDF in the initial treatment of chronically HIV-1-infected individuals may also reduce the risk of HIV transmission from these individuals to their partners (sexually) or from mother to child (perinatally).

Another indication for the clinical use of tenofovir disoproxil fumarate is (lamivudine-resistant) chronic hepatitis B in HIV/HBV-coinfected patients. Several studies have demonstrated that TDF is very effective in reducing HBV DNA levels in HIV/HBV-coinfected patients carrying either wild-type or 3TC-resistant [YMDD variant (rt M204I/V)] HBV.^{85–88} Noteworthy is that TDF treatment for 12 months in patients coinfected with HIV and 3TC-resistant HBV was not associated with the emergence of HIV- or HBV-specific resistance mutations.⁸⁹

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

More than 30 structurally different classes of compounds have been identified as NNRTIs,⁹⁰ namely, compounds that are specifically inhibitory to the replication of HIV-1 (and not any other retroviruses such as HIV-2 or SIV) and that are targeted at a specific, allosteric (i.e., nonsubstrate binding) site of the reverse transcriptase. Three NNRTIs [i.e., nevirapine (Viramune), delavirdine (Rescriptor), and efavirenz (Sustiva, Stocrin)] have so far been formally licensed for clinical use in the treatment of HIV-1 infections. Emivirine (MKC-442)⁹¹ should have been the fourth one, but its development has in the meantime been discontinued. Another NNRTI, the thiocarboxanilide UC-781 (**24**), has been recognized as a retrovirucidal agent capable of reducing the infectivity of HIV-1 virions⁹² and therefore seems to be an ideal candidate microbicide (virucide), i.e., when formulated in replens gel,⁹³ for topical application to prevent the sexual transmission of HIV.



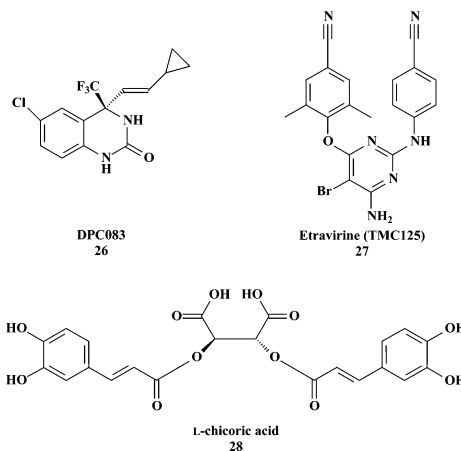
The “first-generation” NNRTIs are notorious for rapidly eliciting virus drug resistance, especially when used singly (as monotherapy). The most common mutations occurring in the clinical setting following the use of NNRTIs are K103N and Y181C. Therefore, attempts have been made to develop “second-generation” NNRTIs that are resilient to such drug resistance mutations. These attempts have led to the identification of a number of compounds that are indeed resilient to the K103N and/or Y181C mutations. Capravirine (**25**) is such a compound that retained activity against HIV-1 strains carrying the K103N mutation in their reverse transcriptase.⁹⁴ Capravirine has proceeded to phase II/III clinical trials, which were temporarily put on hold (because of animal toxicity studies showing vasculitis in dogs after 48 weeks of drug administration) but have recently been resumed.

DPC 083 (**26**), a derivative of efavirenz, has marked activity against HIV-1 strains with various mutations in their reverse transcriptase (L100I, K103N, Y181C, Y188L, K103N + L100I, and K103N + Y181C) that make them resistant to the current NNRTIs.⁹⁵ The long plasma half-life of the compound supports once-daily (or even less frequent) dosing.⁹⁶ Compound **26**, administered orally once-daily at a dose of 100 mg in patients who had failed on the current NNRTIs and harbored NNRTI-resistant mutations, effected a viral load reduction of 1.28 log₁₀ after 8 weeks of treatment.⁹⁷ At 16 weeks, about 40–50% of the subjects had HIV-1 RNA levels below 400 copies per milliliter, suggesting that **26** was active against viral variants with resistance to currently available NNRTIs.⁹⁸

Like **26**, TMC 125 (etravirine) (**27**) is an investigational NNRTI with potent activity against HIV-1 strains resistant to the currently available NNRTIs.⁹⁹ When **27** was administered orally at a dose of 900 mg twice daily for 7 days in treatment-experienced patients with highly NNRTI-resistant virus and failing on an NNRTI-containing regimen, it demonstrated significant antiviral potency (i.e., viral load reduction up to 0.9 log₁₀).¹⁰⁰ In another study, monotherapy with **27** in antiretroviral-naïve HIV-1-infected individuals effected a 1.5–2.0 log₁₀ reduction of viral load after 1 week of treatment, with a similar initial rate of decline of plasma HIV-1 RNA levels as seen with a five-drug regimen.¹⁰¹ While the open-label 7-day phase I/II clinical trial of **27** in HIV-1-infected patients with high-level phenotypic NNRTI-resistant HIV appears very encouraging,¹⁰² longer-term studies seem warranted to verify whether this antiviral potency is sustained and whether **27** does not lead to untoward side effects or engenders drug-resistant mutations on its own.

HIV Integrase Inhibitors

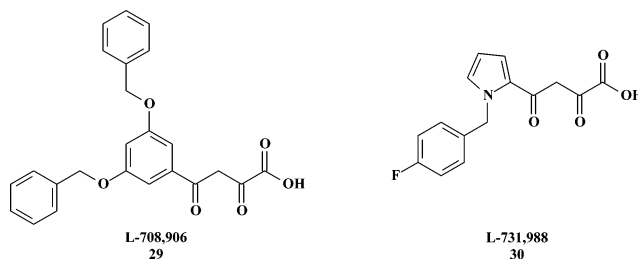
Integration of the proviral DNA into host cell chromosomal DNA is an essential step in the viral replica-



tion cycle. This process is mediated by the viral integrase, and because there is no cellular homologue for this enzyme, it has been considered an attractive target for HIV therapeutics. Numerous small-molecule HIV-1 integrase inhibitors have been described, the two most predominant classes of inhibitors being the catechol-containing hydroxylated aromatics and the diketo acid containing aromatics (for a recent review, see Dayam and Neamati ref 103).

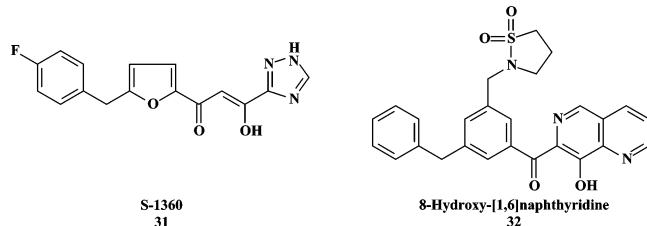
An example from the first class of compounds is L-chicoric acid (**28**). Integrase was identified as the molecular target for the action of L-chicoric acid, since a single amino acid substitution (G140S) in the integrase rendered the corresponding HIV-1 mutant resistant to L-chicoric acid.¹⁰⁴ It was later shown that the G140S mutation confers resistance not only to L-chicoric acid but also to the diketo acid L-731,988 and furthermore attenuates the catalytic activity of the enzyme.¹⁰⁵ In our hands, however, HIV-1 integrase carrying the G140S mutation appeared to be as sensitive to the inhibitory effect of L-chicoric acid as the wild-type integrase.¹⁰⁶ Upon repeated passages of the virus in the presence of L-chicoric acid, mutations were found in the viral envelope glycoprotein gp120 but not in the integrase; HIV strains resistant to polyanionic compounds showed cross-resistance to L-chicoric acid, and time-of-addition experiments further confirmed that the primary site of interaction for L-chicoric acid is the virus adsorption stage rather than the integrase.¹⁰⁶

The structure of the HIV-1 integrase core domain complexed with the inhibitor 5CITEP [1-(5-chloroindol-3-yl)-3-hydroxy-3-(2*H*-tetrazol-5-yl)propenone] has been described as a platform for the structure-based design of novel HIV-1 integrase inhibitors.¹⁰⁷ This was followed by the description of a number of diketo acids such as L-708,906 (**29**) and L-731,988 (**30**) as inhibitors of the integrase-mediated strand transfer reaction (which is responsible for the covalent linkage of the viral DNA 3' ends to the cellular DNA).¹⁰⁸ The diketo acid derivatives



29 and **30** were also found to inhibit HIV-1 replication in cell culture, and addition of **29** could be postponed without loss of activity till 7 h after infection, a time point that coincides with (the onset of) proviral DNA integration.¹⁰⁹ That the proviral DNA integration is indeed the target of action for the diketo acids in cell culture could be confirmed by quantitative Alu-PCR.¹⁰⁹ Repeated passages of HIV-1 in the presence of **29** led to the successive accumulation of three mutations, i.e., T66I, L74M, and S230R, in the integrase.¹¹⁰ The triple-mutant virus (T66I/L74M/S230R) showed a 10-fold reduced susceptibility to **29** and cross-resistance to the diketo acid derivative S-1360 but remained fully sensitive to the pyranodipyrimidine V-165, another integrase inhibitor type.¹¹⁰

S-1360 (**31**) actually represents the first integrase inhibitor to reach clinical studies.¹¹¹ Compound **31** would inhibit the HIV-1 integrase at an IC₅₀ of 20 nM and the HIV-1 replication at an EC₅₀ of 140 nM while its CC₅₀ would be 110 μM, thus achieving a therapeutic index of almost 1000, the highest selectivity thus far reported for an integrase inhibitor. Compound **31** is orally bioavailable and phase I/II studies with **31** in HIV-infected subjects have been initiated.¹¹¹ The mechanism of action of the diketo acids (i.e., **29** and **30**), 5CITEP, and **31** may be based on an interaction between the carboxylate group of the diketo acids or the isosteric heterocycle in the two other compounds and metal ion(s) in the active site of the integrase, resulting in a functional sequestration of these critical metal cofactors.¹¹²



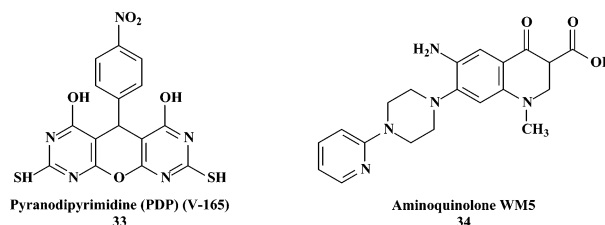
Diketo acids could be classified into two groups: those similarly potent in the presence of magnesium and manganese and those potent in manganese and relatively ineffective in the presence of magnesium. Both the aromatic and the carboxylic or tetrazole functions of the diketo acids determine their metal-chelating selectivity.¹¹³

Starting from **30**, several additional 4-aryl-2,4-dioxobutanoic acid derivatives have been described as HIV-1 integrase inhibitors.¹¹⁴ Also, new azido-containing aryl diketo acids have been described as HIV-1 integrase inhibitors capable of conferring antiviral protection in HIV-infected cells.¹¹⁵ Interestingly, from **30** a novel diketo acid, 4-[5-(benzoylamino)thien-2-yl]-2,4-dioxobutanoic acid, has been derived that binds to and inhibits the RNase H (p15) domain of the HIV-1 reverse transcriptase,¹¹⁶ the metal-dependent behavior of this RNase H inhibitor being analogous to the mechanism of action proposed for HIV-1 integrase inhibitors of the same structural class.¹¹²

Inhibition of the strand transfer reaction of the integration process has been reported for 8-hydroxy[1,6]-naphthyridines. The naphthyridine shown (**32**) inhibits strand transfer catalyzed by integrase with an IC₅₀ of

10 nM and inhibits 95% of the spread of HIV-1 infection in cell culture at 0.39 μM, while no cytotoxicity is exhibited in cell culture at ≤12.5 μM, and a good pharmacokinetic profile was displayed when dosed orally to rats.¹¹⁷

Recently, an entirely new class of HIV integrase inhibitors was identified, namely, that of the 5*H*-pyrano[2,3-*d*:6,5-*d'*]dipyrimidines (PDPs). The most potent congener of this series, 5-(4-nitrophenyl)-2,8-dithiol-4,6-dihydroxy-5*H*-pyrano[2,3-*d*:6,5-*d'*]dipyrimidine (V-165) (**33**), inhibited the replication of HIV-1 at an EC₅₀ of 8.9 μM, which is 14-fold below the cytotoxicity threshold.¹¹⁸ A close correlation was found between the anti-HIV activity observed in cell culture and the inhibitory activity in the integrase strand transfer assays. Time-of-addition experiments indicated that **33** interfered with the viral replication cycle at a time point coinciding with proviral DNA integration, a conclusion that was corroborated by quantitative Alu-PCR. Yet, at the molecular level, the mechanism of action of **33** must be different from that of the diketo acids, since the mutations (T66I, L74M, and S230R) that engendered resistance to the diketo acids did not do so toward **33**.¹¹⁰



Transcription (Transactivation) Inhibitors

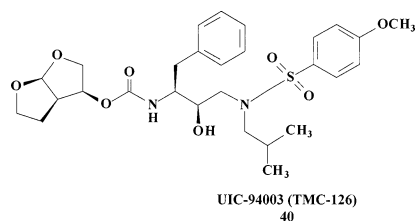
At the transcriptional level, HIV gene expression may be inhibited by compounds that interact with cellular factors (such as NF-κB) that bind to the LTR promoter and that are needed for basal-level transcription. Greater specificity, however, may be expected from those compounds that specifically inhibit the transactivation of the HIV LTR promoter by the viral Tat (trans-activating) protein.¹¹⁹ A number of compounds have been reported to inhibit HIV-1 replication in both acutely and chronically infected cells, through interference with the transcription process that could at least partially be attributed to inhibition of Tat (or other transactivators): K37 [7-(3,4-dehydro-4-phenyl-1-piperidinyl)-1,4-dihydro-6-fluoro-1-methyl-8-trifluoromethyl-4-oxoquinoline-3-carboxylic acid];¹²⁰ temacrazine [1,4-bis(3-(6-oxo-6*H*-*v*-triazolo[4,5,1-*de*]acridin-5-yl-aminopropyl)piperazine];¹²¹ flavopiridol, a cyclin-dependent kinase (CdK) inhibitor;¹²² EM2487, a natural product from *Streptomyces*;¹²³ and CGP 64222, a 9-mer peptoid structurally reminiscent of the amino acid 48–56 sequence RKKRRQRRR of Tat.¹²⁴ Further analysis, however, revealed that the peptoid CGP 64222 owes its anti-HIV activity in cell culture primarily to an interaction with CXCR4, the coreceptor for X4 HIV strains.¹²⁵

A 6-aminoquinolone, WM5 (**34**), was recently shown to inhibit HIV-1 replication in acutely infected as well as chronically infected cells. This aminoquinolone was found to efficiently bind to TAR RNA and to inhibit Tat-mediated LTR-driven transcription. Compound **34** may be a promising lead compound for the development of a new class of HIV-1 transcription inhibitors.¹²⁶

I84V) virus, selected upon repeated passage of the virus in the presence of the compound, remained sensitive to saquinavir but showed various levels of cross-resistance to nelfinavir, indinavir, ritonavir, and amprenavir.¹³⁴ Atazanavir appeared to have a resistance profile that is distinct from that of the other PIs. Analysis of the genotype profiles of 943 PI-susceptible and -resistant clinical isolates identified a strong correlation between the presence of amino acid changes at specific residues (10I/V/F, 20R/M/I, 24I, 33I/F/V, 36I/L/V, 46I/L, 48V, 54V/L, 63P, 71V/T/I, 73C/S/T/A, 82A/F/S/T, 84V, and 90M) and decreased susceptibility to atazanavir. While no single substitution or combination of substitutions was predictive of atazanavir resistance (>3.0-fold reduction in susceptibility), the presence of at least five of these substitutions correlated strongly with loss of atazanavir susceptibility.¹³⁵

In highly antiretroviral-experienced patients, once-daily atazanavir/saquinavir showed comparable efficacy to twice-daily ritonavir/saquinavir, both in combination with two NRTIs.¹³⁶ In another study, once-daily atazanavir (200, 400, or 500 mg) was compared with nelfinavir (750 mg three times daily) when given both as monotherapy and in combination with didanosine and stavudine in antiretroviral-naïve subjects infected with HIV-1. Once-daily atazanavir rapidly and durably suppressed HIV RNA levels and durably increased CD4 cell counts. Through 48 weeks, atazanavir was not associated with clinically relevant increases in total cholesterol, fasting LDL cholesterol, or fasting triglycerides. In comparison, nelfinavir led to prompt, marked, and sustained elevations in these parameters of a magnitude that suggests they are clinically relevant.¹³⁷ Other studies have ascertained that atazanavir, compared to efavirenz (both combined with fixed dose zidovudine and lamivudine), in antiretroviral-naïve patients, produced a comparable and proportional effect on body fat distribution at 48 weeks, consistent with weight gain and not with the patterns of central adiposity characteristic of the development of lipodistrophy.¹³⁸

Under clinical development is TMC 114, a structural analogue of TMC 126 (UIC-94003) (**40**). Compound **40**



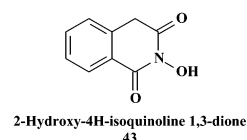
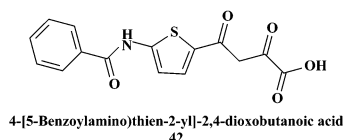
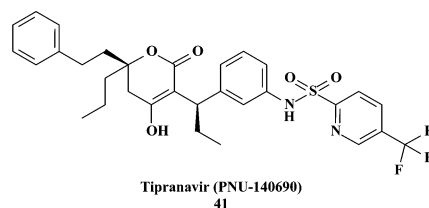
is a peptidomimetic PI containing a bis-tetrahydrofuran urethane and 4-methoxybenzenesulfonamide (and thus structurally related to amprenavir). It was reported to be extremely potent against a wide spectrum of HIV-1 strains ($EC_{50} = 0.3\text{--}0.5$ nM), whether resistant to other PIs or not.¹³⁹ Upon passage of HIV-1 in the presence of **40**, mutants carrying a novel active-site mutation, A28S, emerged. Modeling studies revealed close contact of **40** with the main chain of the protease active-site amino acid residues D29 and D30 different from that of the other PIs, and this may be important for its potency, particularly against drug-resistant HIV-1 variants.¹³⁹ TMC 114, like TMC 126, has an excellent activity profile

against HIV variants that are highly resistant to current PIs.¹⁴⁰ Clinical studies with TMC 114 were initiated some time ago.¹⁴¹ TMC 114 boosted with ritonavir (TMC 114/rvt), when given for 14 days, proved effective in reducing viral load by 1.38 \log_{10} in multiple PI-experienced patients with PI resistance-associated mutations.¹⁴²

Tipranavir (PNU-140690) (**41**) is a nonpeptidomimetic inhibitor of the HIV protease,^{143,144} which, as could be expected, shows little cross-resistance to the peptidomimetic PIs. Of 105 clinical HIV-1 isolates with more than 10-fold reduced susceptibility to three or four of the peptidomimetic PIs and an average of six mutations per isolate, 90% were fully susceptible, 8% had 4- to 10-fold resistance, and only 2% had more than 10-fold resistance to tipranavir.¹⁴⁵ Tipranavir retained marked activity against HIV-1 isolates derived from patients with multidrug resistance to other PIs;¹⁴⁶ it might therefore be useful in drug combination regimens with other antiretroviral agents for patients who already failed on other PI-containing drug regimens. Tipranavir drug levels can be markedly increased (“boosted”) by coadministration with ritonavir.¹⁴⁷ In a study of patients experiencing virological failure on their second PI-containing regimen, subjects were randomized to receive tipranavir (500 or 1000 mg)/ritonavir (100 mg) twice daily. At 48 weeks in the lower-dose group, the mean change in HIV RNA was $-2.4 \log_{10}$ copies per milliliter for subjects with five or fewer PI-associated resistance mutations versus $-2.2 \log_{10}$ copies per milliliter with greater than five PI-associated mutations.¹⁴⁸ Further clinical investigations with tipranavir are in progress.

HIV Ribonuclease H Inhibitors

During the reverse transcription process that converts the single-stranded viral RNA into double-stranded (pro)viral DNA, a RNA·DNA heteroduplex (hybrid) is formed. The RNA strand of this heteroduplex must be cleaved by the ribonuclease H (RNase H) component (p15 domain of the p66 subunit of the p66/p51 reverse transcriptase heterodimer) before the remaining (–)DNA strand can be duplicated to give the double-stranded (pro)viral DNA that is then integrated into the host cell genome. RNase H has been considered as an attractive target for the development of new antiretroviral agents, and indeed, several recent reviews have addressed the possibility of inhibiting RNase H activity by small-molecule inhibitors.^{149–151}



It appears feasible to specifically inhibit HIV-1 RNase H by, for example, novel diketo acids,¹⁵² such as 4-[5-

(benzoylamino)thien-2-yl]-2,4-dioxobutanoic acid (**42**), and *N*-hydroxyimides,^{153,154} such as 2-hydroxy-4*H*-isoquinolone-1,3-dione (**43**). In both cases, binding of the compound was (much) stronger in the presence of Mn²⁺ compared to Mg²⁺. Such metal dependence is characteristic of the diketo acid type of HIV integrase inhibitors (see above), and not surprisingly, compound **42** was also found to inhibit HIV-1 integrase in vitro at roughly the same concentration at which it was found to inhibit RNase H activity.¹⁵² Whether compounds (related to **42** and **43**) are able to inhibit HIV replication in cells remains to be established. Compound **42** was evaluated (and found inactive) against HIV-1 replication in cell culture.¹⁵²

Conclusion

In recent years, considerable progress has been made toward the chemotherapy of HIV infections. New compounds have been licensed for clinical use: the virus entry inhibitor enfuvirtide (T-20), the NRTI emtricitabine [(–)-FTC], the NtRTI tenofovir disoproxil fumarate (TDF), the PI atazanavir, and the fixed dose combination of TDF with (–)-FTC. Other compounds have further proceeded through preclinical and/or clinical evaluation: CXCR4 antagonists (i.e., AMD070) and CCR5 antagonists (i.e., SCH-C), new NRTIs such as amdoxovir, new NNRTIs such as etravirine (TMC 125), integrase inhibitors such as S-1360, and new PIs such as tipranavir. Yet other, new anti-HIV agents acting at new targets by novel mechanisms (which have been, or still remain to be, elucidated) have been identified: CD4 down-modulators (i.e., cyclotriazadisulfonamides), gp120 binders (i.e., plant lectins, glycopeptide antibiotics), HIV integrase inhibitors such as the pyranodipyrimidine V-165, and two new classes of compounds (i.e., *N*-aminoimidazoles and pyridine oxide derivatives) that seem to interfere with a postintegration, transcription transactivation event that remains the subject of further investigation.

A major breakthrough at the clinical stage is that now several once-daily dose regimens have become available for the new compounds tenofovir disoproxil fumarate, emtricitabine, and atazanavir, as well as for some of the “older” compounds such as efavirenz and didanosine. Preliminary randomized clinical trials have shown that these once-a-day regimens provide a virological efficacy that is at least similar to that of conventional HAART (“highly active antiretroviral therapy”).¹⁵⁵ In addition, the once-daily dosing is likely to be accompanied by increased tolerability and compliance. Is there still room for improvement? Recent studies have indicated that novel combinations of lopinavir/ritonavir, efavirenz, tenofovir disoproxil fumarate, and lamivudine, compared to matched cohorts with alternative combination regimens, may still augment the potency of HAART by as much as 25–30%.¹⁵⁶

It is evident that in the future, as in the past, the greatest benefit, especially in terms of enhanced (synergistic) potency and reduced risk of virus-drug resistance development, may be expected from multiple (for example, triple) drug combination regimens consisting of different drugs targeting different viral proteins (or different sites of the same protein). However, while at the cellular level drug combinations often lead to a

synergistic action and diminish the likelihood of resistance development, in vivo drug combinations should be carefully monitored for possible pharmacokinetic drug–drug interactions, which for the individual drugs may necessitate dose adjustments to generate the optimal drug plasma levels.

Which (triple) drug regimen would at the present time be advocated? As mentioned above, the combination of TDF with (–)-FTC and efavirenz seems to be one of the more promising drug combinations. Certainly, triple-nucleoside combination of abacavir (ABC), zidovudine (AZT), and lamivudine (3TC) should not be favored over regimens containing efavirenz and two or three nucleoside analogues.¹⁵⁷ In addition, the triple-nucleoside (ABC, 3TC, and AZT) regimen carries a risk of virologic failure.¹⁵⁸ Antiretroviral therapy with efavirenz or nevirapine showed similar efficacy, so triple-drug regimens with either NNRTI are valid for first-line treatment.¹⁵⁹

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Biography

Erik De Clercq, M.D., Ph.D., is Chairman of the Department of Microbiology and Immunology of the Katholieke Universiteit Leuven (Belgium), President of the Rega Foundation, and Chairman of the Directory Board of the Rega Institute for Medical Research. As a Full Professor since 1977, he is teaching the courses in cell biology, biochemistry, microbiology, and virology at the K. U. Leuven Medical School. He is a titular member of the Belgian Royal Academy of Medicine and the Academia Europaea. Prof. De Clercq's scientific achievements are in the antiviral chemotherapy field. He has (co)discovered a number of drugs for the treatment of virus infections: herpes simplex (valaciclovir), herpes zoster (brivudin), AIDS (stavudine), cytomegalovirus (cidofovir), hepatitis B (adefovir), and HIV (AIDS) (tenofovir disoproxil fumarate, marketed as Viread, and, in combination with emtricitabine, as Truvada).

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