Topics in Medicinal Chemistry 15

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Therapy of Viral Infections

Springer

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Editors

Therapy of Viral Infections

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15
Topics in Medicinal Chemistry

Aims and Scope

Drug research requires interdisciplinary team-work at the interface between chemistry, biology and medicine. Therefore, the new topic-related series Topics in Medicinal Chemistry will cover all relevant aspects of drug research, e.g. pathobiochemistry of diseases, identification and validation of (emerging) drug targets, structural biology, drugability of targets, drug design approaches, chemogenomics, synthetic chemistry including combinatorial methods, bioorganic chemistry, natural compounds, high-throughput screening, pharmacological in vitro and in vivo investigations, drug-receptor interactions on the molecular level, structure-activity relationships, drug absorption, distribution, metabolism, elimination, toxicology and pharmacogenomics.

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In references Topics in Medicinal Chemistry is abbreviated Top Med Chem and is cited as a journal.

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Preface

Even though many viral infections can meanwhile be prevented by prophylactic vaccination, there is still urgent need for the development of new antiviral agents tackling contemporary afflictions of mankind such as HIV, Dengue Fever, West-Nile-Fever, or influenza, to name just a few, as for those infections either no vaccination exits or has to be reformulated annually due to antigenic drift and shift of the virus as in case of influenza.

In the last decades tremendous progress in the development of antiviral agents has been made resulting, e.g., in case of HIV, not only in a significant prolongation but also in an improvement of the quality of the patient's life. Nevertheless, development of resistance towards an approved drug often hampers the clinical use and might in the worst case result in a lack of treatment options for certain patients. Thus, not only the continuous development of new drugs but also the identification and validation of novel drug targets is of utmost importance to ensure that these infections can be adequately treated.

This special issue of *Topics in Medicinal Chemistry* "Therapy of viral infections" focuses on the one hand on recent developments of inhibitors against well-established drug targets and on the other hand new targets on either the virus or its host side are presented.

The issue starts with a review by Demeulemeester, De Maeyer, and Debyser on HIV-1 integrase inhibitors spanning from the first approved inhibitors to recent developments in this intriguing field. In the following, Macchi, Romeo, Chiacchio, Frezza, Giofré Marino-Merlo, and Mastino highlight in their contribution the development of phosphonated nucleoside analogues starting from the well-known nucleoside analogues such as AZT towards the newer derivatives of acyclic nucleoside phosphonates and phosphonated carbocyclic nucleosides.

In the following Steuber, Kanitz, Ehlert, and Diederich provide a comprehensive review of recent advances targeting the Dengue and West-Nile Protease utilizing small molecule inhibitors. Steinmetzer, Hardes, Böttcher-Friebertshäuser, and Garten focus in their contribution on strategies for the development of influenza drugs. Besides approved drugs and their further development, especially alternative strategies such as inhibition of the fusion and the uncoating process, as well as inhibition of HA-activating host proteases and additional innovative strategies that interfere with host mechanisms, are discussed in detail. Finally, Dürr, Keppler,

Christ, Crespan, Garbelli, Maga, and Dietrich provide a comprehensive review on targeting cellular co-factors in HIV therapy, which might offer new opportunities to interfere with virus replication and, moreover, reduce the development of drug resistance.

We are grateful to all authors of this special issue of *Topics in Medicinal Chemistry* "Therapy of viral infections" for the valuable contributions and also would like to thank all reviewers for their very constructive comments.

In addition, we thank the series editors Bernstein, Buschauer, Georg, Lowe, Stilz, Supuran, and Saxena for the invitation and the opportunity to compile this special issue. Finally, we would like to acknowledge the Springer publishing editor Wassermann and the project coordinator Jaeger for the smooth handling of technical aspects related to the special issue.

Berlin, Germany — Holger Steuber
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Contents

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HIV-1 Integrase Drug Discovery Comes of Age

Jonas Demeulemeester, Marc De Maeyer, and Zeger Debyser

Abstract Insertion of the viral genome into host cell chromatin is a pivotal step in the replication cycle of the human immunodeficiency virus and other retroviruses. Blocking the viral integrase enzyme that catalyzes this reaction therefore provides an attractive therapeutic strategy. Nevertheless, many years lie between the initial discovery of integrase and the clinical approval of the first integrase strand transfer inhibitor, raltegravir, in 2007. Recently, elvitegravir was second to make it into the clinic, while dolutegravir, a second-generation integrase inhibitor, is close to receiving the green light as well. Viral resistance and cross-resistance among these strand transfer inhibitors however warrant the search for compounds targeting HIV integration through different mechanisms of action. The most advanced class of allosteric integrase inhibitors, coined LEDGINs or non-catalytic integrase inhibitors (NCINIs), has shown remarkable antiviral activity that extends beyond the viral integration step. Time will tell however if they will stand the test of clinical development. Notably, the development of LEDGINs and other integrase inhibitors is aided by recent structural and mechanistic insights into the retroviral integration apparatus. Here we provide an overview of the development of integrase strand transfer and allosteric inhibitors while exploring their mechanisms of action and patterns of viral resistance.

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Keywords Allosteric, HIV, LEDGF/p75–IN interaction, Non-catalytic site integrase inhibitor, Strand transfer

Contents

Abbreviations

1-LTR	1-Long terminal repeat
2-LTR	2-Long terminal repeats
3P	3′-Processing
ADME	Absorption distribution, metabolism, and excretion
AIDS	Acquired immunodeficiency syndrome
ALLINI	Allosteric integrase inhibitor
ART	Antiretroviral therapy
ARV	Antiretroviral
CC_{50}	50% cytotoxic concentration
CCD	Catalytic core domain
CCR5	C–C chemokine receptor 5
CR	Charged region
CTD	C-terminal domain
DKA	Diketo acid
DNA	Deoxyribonucleic acid
EC_{50}	50% Effective concentration
FDA	Food and Drug Administration
HAART	Highly active antiretroviral therapy
HCV	Hepatitis C virus

HDGF	Hepatoma-derived growth factor
HIV-1	Human immunodeficiency virus type 1
HIV-2	Human immunodeficiency virus type 2
HRP-2	Hepatoma-derived growth factor-related protein 2
HSAB	Hard and soft Lewis acids and bases
IBD	Integrase-binding domain
IC_{50}	50% inhibitory concentration
IN	Integrase
INI	Integrase inhibitor
IRBM	Instituto di Ricerche di Biologia Moleculare
LEDGF/p75	Lens epithelium-derived growth factor/p75
LTR	Long terminal repeat
MACCS	Molecular access system
mRNA	Messenger ribonucleic acid
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NCINI	Non-catalytic site integrase inhibitor
NLS	Nuclear localization signal
NMR	Nuclear magnetic resonance
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
NTD	N-terminal domain
$PAEC_{50}$	Protein-adjusted 50% effective concentration
$PAIC_{50}$	Protein-adjusted 50% inhibitory concentration
PFV	Prototype foamy virus
PHAT	Pseudo-HEAT analogous topology
PIC	Pre-integration complex
PK	Pharmacokinetic
PPI	Protein–protein interaction
PR	Protease
PrEP	Preexposure prophylaxis
PWWP	Pro–Trp–Trp–Pro domain
RNA	Ribonucleic acid
RT	Reverse transcriptase
SAR	Structure–activity relationship
SIV	Simian immunodeficiency virus
SPR	Surface plasmon resonance
ST	Strand transfer
STD-NMR	Saturation transfer difference nuclear magnetic resonance
tDNA	Target deoxyribonucleic acid
TRN-SR2	Transportin-SR2
vDNA	Viral deoxyribonucleic acid
WT	Wild type

1 Introduction

Human immunodeficiency virus type 1 (HIV-1) is a single-stranded, positive RNA virus of the genus *Lentivirinae*. Its roots can be traced back to Africa to at least four individual zoonotic infections with simian immunodeficiency viruses (SIV) from gorilla and chimpanzee [1]. A disease was however not recognized until the first reports of clustered cases of *Pneumocystis carinii* pneumonia [2] and Kaposi's sarcoma [3] appeared in 1981. It took two more years before the research groups of Montagnier and Gallo isolated a new virus, dubbed lymphadenopathy-associated virus by Montagnier [4] and human T-lymphotropic virus III by Gallo [5], which they suggested to be the etiological agent of a disease known by that time as acquired immunodeficiency syndrome (AIDS). The virus obtained in the two studies was shown to be the same and was renamed human immunodeficiency virus (HIV) in 1986. Today, over 34 million people from all over the world are living with the virus and over 30 million have already passed away. Even though the number of new infections globally is on the decline, it was still estimated at 2.5 million in 2011 [6].

The success of HIV-1 stands in shrill contrast to its apparent simplicity. Its small 9.7 kb genome encodes a mere 15 mature proteins which allow it to elegantly manipulate the infected cell and subvert the innate and adaptive immune responses of the host, resulting in a persistent infection in humans. Viral replication proceeds through a number of characteristic steps, depicted in Fig. 1 (reviewed in [7]). Briefly, after attachment and membrane fusion, the viral core enters the cell and uncoats while the reverse transcriptase (RT) creates a double-stranded DNA copy of the viral genome and a pre-integration nucleoprotein complex (PIC) is formed. The PIC is imported into the nucleus where integrase (IN) catalyzes insertion of the viral DNA (vDNA) into the host chromatin, irreversibly establishing a provirus in the host cell. Transcription and translation take place, giving rise to viral proteins that assemble into budding virions at the cell membrane. Released particles undergo proteolytic maturation by the viral protease (PR), producing novel infectious virions. Each of these steps represents a potential target for antiretroviral therapy (ART). Notably, RT and PR have thus far been targeted most extensively. Clinical use of their inhibitors has markedly improved patient survival and delayed progression to AIDS, resulting in the present-day decrease in incidence as well as in HIV-/AIDS-related mortality.

Since the identification of the first antiviral for clinical use against AIDS, zidovudine (3′-azido-2′,3′-dideoxythymidine, AZT) in 1985, ART has evolved significantly (reviewed in [8]). Treatment developed from AZT monotherapy in the late 1980s over dual therapy of AZT plus zalcitabine (2′,3′-dideoxycytidine, ddC) to the current standard of care, triple therapy. This combination therapy includes at least three antiviral compounds and aims for high synergy, low toxicity, and reduced resistance development. As such, it has come to be known as highly active antiretroviral therapy (HAART) [8]. Thirty years of research and clinical translation have endowed us with a repertoire of 25 US Food and Drug Administration (FDA)-approved compounds,

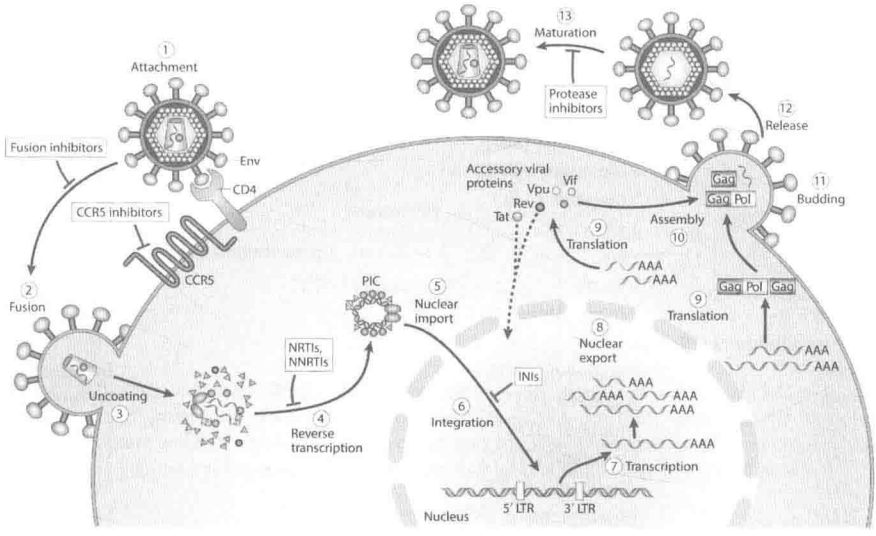

Fig. 1 Replication cycle of HIV-1. After Env-mediated attachment (*1*) of the virus to the cell via the CD4 receptor and binding to the CXCR4 or CCR5 coreceptor, fusion (*2*) of the viral membrane and the cell membrane is initiated. This releases the conical capsid into the cytoplasm where uncoating (*3*) takes place. In the meantime reverse transcriptase creates a double-stranded DNA copy of the viral genome (*4*), which associates with various viral and cellular proteins to form the pre-integration complex (PIC). This nucleoprotein complex is actively imported (*5*) into the nucleus where integrase catalyzes insertion (*6*) of the viral DNA into the host chromatin. Proviral transcription (*7*) and export (*8*) of viral mRNA allow for translation (*9*) of new viral proteins that in turn assemble (*10*) into budding particles (*11*) at the cell membrane. These particles are released (*12*) from the cell and mature (*13*) through polyprotein processing by the viral protease, completing the viral replication cycle. Steps that are targeted in antiretroviral therapy are indicated. CCR5, C-C chemokine receptor 5; NRTI, nucleoside reverse transcriptase inhibitors; NNRTI; non-nucleoside reverse transcriptase inhibitors; INIs, integrase inhibitors; LTR, long terminal repeat. Adapted by permission from Macmillan Publishers Ltd: Nat Rev Microbiol [7], copyright 2012

falling into six distinct classes based on the viral target, which represent the building blocks for HAART (Table 1).

Irrespective of this seemingly large armamentarium of drugs, HIV's vast evolutionary potential allows for rapid emergence of resistant variants. As no cure is available and lifelong treatment is required, the door is always open to resistance development. Additionally, an effective vaccine remains beyond our grasp and hence research efforts focused on exploring novel drug targets in retroviral biology are critical to control the HIV/AIDS pandemic. The main retroviral target that was clinically unexplored until 2007 is the third viral enzyme, IN. Indeed, it is evident from Table 1 that inhibitors of the viral RT or PR far outnumber and predate those targeting IN. There are two main reasons for this. First, for many years a relevant high-throughput screen for IN inhibitors was lacking, and few of the compounds that showed inhibition in vitro retained

Table 1 Antiretrovirals currently approved by the FDA

Approval date	Generic name	Brand name
Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs)		
1986	Zidovudine (ZDV or AZT)	Retrovir
1991	Didanosine (ddI)	Videx
1992	Zalcitabine (ddC)[a]	Hivid
1994	Stavudine (d4T)	Zerit
1995	Lamivudine (3TC)	Epivir
1998	Abacavir (ABC)	Ziagen
2001	Tenofovir disoproxil fumarate (TDF)	Viread
2003	Emtricitabine (FTC)	Emtriva
Protease inhibitors (PIs)		
1995	Saquinavir (SQV)	Invirase
1996	Ritonavir (RTV)	Norvir
1996	Indinavir (IDV)	Crixivan
1997	Nelfinavir (NFV)	Viracept
2003	Atazanavir (ATV)	Reyataz
2003	Fosamprenavir (FPV)	Lexiva
2005	Tipranavir (TPV)	Aptivus
2006	Darunavir (DRV)	Prezista
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)		
1996	Nevirapine (NVP)	Viramune
1997	Delavirdine (DLV)	Rescriptor
1998	Efavirenz (EFV)	Sustiva
2008	Etravirine (ETR)	Intelence
2011	Rilpivirine (RPV)	Edurant
Fusion inhibitors (FIs)		
2003	Enfuvirtide (T-20)	Fuzeon
CCR5 antagonists		
2007	Maraviroc (MVC)	Selzentry
Integrase inhibitors (INIs)		
2007	Raltegravir (RAL)	Isentress
2012	Elvitegravir (ELV)[b]	

[a]Zalcitabine is no longer marketed

[b]At present elvitegravir is only approved as part of the fixed-dose combination Stribild

their effect when assayed on viral replication. Second, although many nuclear magnetic resonance (NMR) and crystal structures (X-ray) for all three domains of HIV-1 IN were solved [9], structural information on the full-length protein and its catalytic assemblies was lacking. Together, these elements rendered IN drug discovery effectively blind for many years. This chapter looks at how integrase drug discovery has recently come of age with the development of two potent inhibitor classes and crucial new insights into the functioning of the enzyme itself. The results do not only epitomize scientific breakthroughs, but represent vital milestones to maintain the success of HAART in the future.

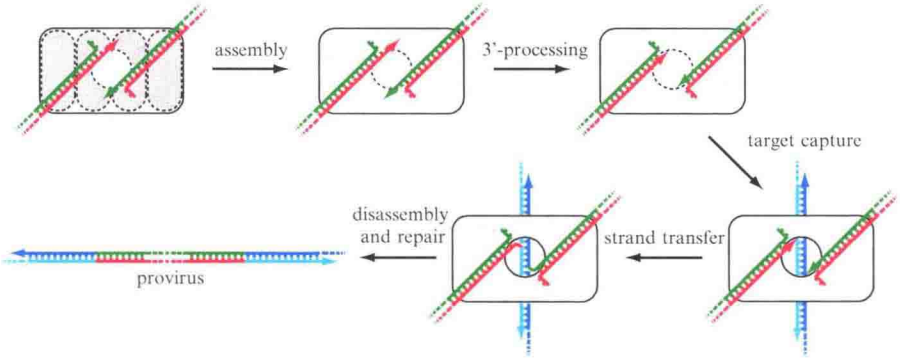

Fig. 2 Schematic overview of the integration process. IN assembles on the vDNA ends and catalyzes the 3′-processing reaction. The tetrameric IN–vDNA assembly known as the intasome can then capture a tDNA strand and carry out strand transfer. Disassembly of the complex and repair of the gapped intermediate by cellular enzymes results in a stably integrated provirus

2 Integration

After nuclear import of the PIC, the viral genome is inserted into host chromatin. Viral IN is the central player here, catalyzing the essential DNA cutting and joining reactions (represented schematically in Fig. 2 and reviewed in [10]). Briefly, following its assembly with the vDNA, IN removes a dinucleotide from both 3′ ends, a step known as 3′-processing (3P). After capture of the host target DNA (tDNA) a tetrameric IN complex with vDNA, the intasome, catalyzes the strand transfer (ST) reaction with the recessed ends. The result is a gapped intermediate where both vDNA strands are joined by their 3′ ends to opposing tDNA strands. The insertion sites are separated from each other by five base pairs in the case of HIV. The gaps are repaired by host cell repair machinery and a provirus is established.

2.1 Integrase: Structure and Function

All retroviral INs contain three structurally conserved domains, an N-terminal, a catalytic core, and a C-terminal domain (NTD, CCD, and CTD, respectively, Fig. 3; see [9–11] for recent structural reviews). The three canonical domains are connected through flexible linkers and are all essential for the biological activity of the enzyme. The NTD is a three-helix bundle stabilized by coordination of a Zn^{2+} ion through its His_2-Cys_2 (HH-CC) zinc-binding motif (Fig. 3a, b) [12, 13]. The CCD embodies the catalytic heart of the enzyme and bears a DD-X_{35}-E motif characteristic of the DDE(D) nucleotidyltransferase family, which includes various transposases as well as RNase H [14]. The signature DDE(D) residues are brought together in the tertiary structure at the active site where they form a triad

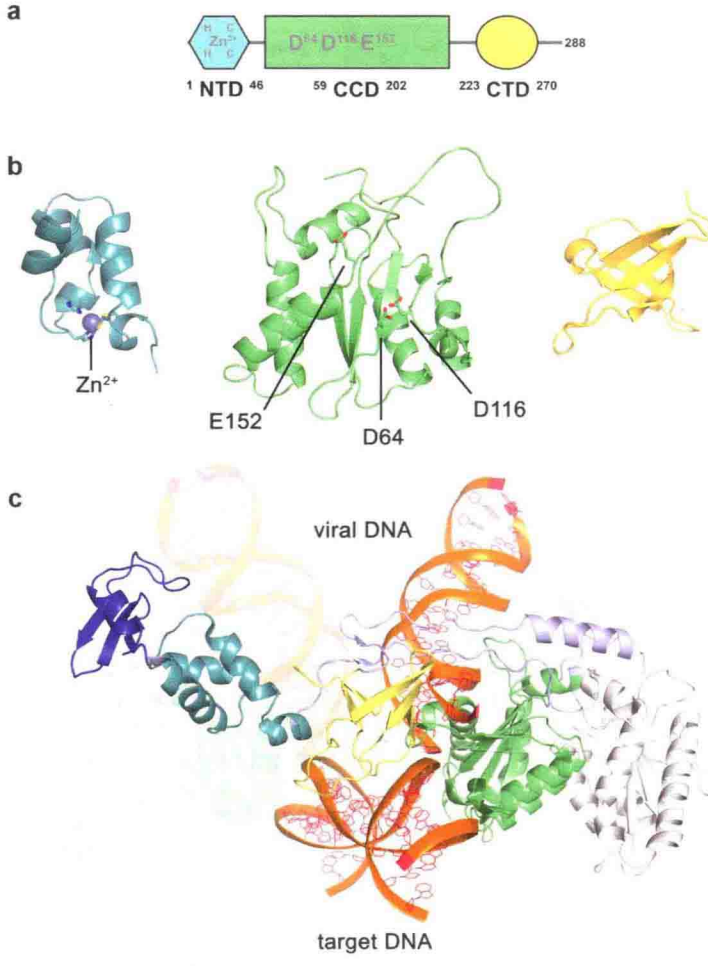

Fig. 3 Structure of retroviral integrase. (**a**) Domain structure and boundaries of HIV-1 integrase. (**b**) From left to right, tertiary structures of the N-terminal [12, 13], catalytic core [15], and C-terminal [16] domains (NTD, CCD, and CTD, respectively). The HH-CC Zn^{2+} binding site and the DDE catalytic triad residues are indicated. (**c**) Structure of the prototype foamy virus (PFV) intasome [18]. One of the two symmetry-related integrase dimers in the assembly is rendered transparent for clarity. The inner monomers containing the two active sites of the complex are color-coded as in (**a**) and (**b**), while the outer supporting monomers are *grey*. The two oligos representing viral DNA ends can be seen to enter the complex from the top while the target DNA lies perpendicular to the paper. PFV integrase contains an additional N-terminal extension domain (NED, colored *deep blue*) on its N-terminus, which is not present in HIV-1 [17]

of carboxylates that coordinate two metal ions (Mg^{2+} or Mn^{2+}) essential for the enzyme's catalytic function (Fig. 3a, b) [15]. The CTD folds into a five-strand β-barrel which is reminiscent of a Src homology 3 domain (SH3, Fig. 3a, b) [16]. All domains are involved in DNA binding and multimerization but the CCD harbors the entire active site.

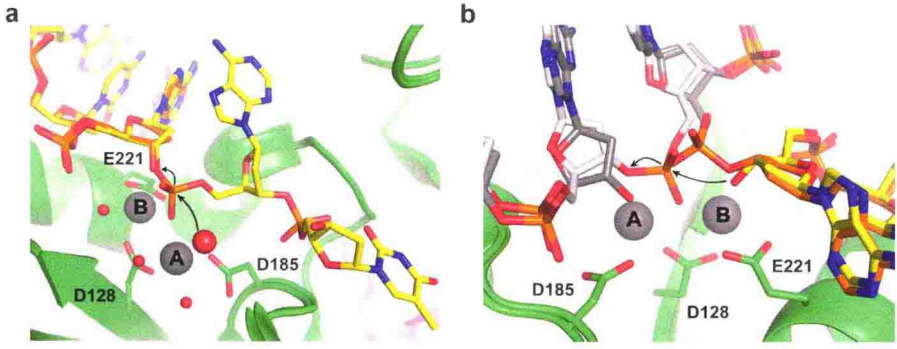

Fig. 4 Integrase catalysis: superposition of the intasome active site before and after (**a**) 3′-processing and before and after (**b**) strand transfer [17, 19]. PFV IN catalytic triad residues D128, D185, and E221 as well as Mg^{2+} ions A and B are indicated. The viral DNA before and after each reaction is shown in *yellow* and *orange*, respectively. Target DNA in (**b**) is shown in *light* and *dark grey*, respectively, before and after

As stated above, full-length IN has proven refractory to structural studies. Fortunately though, the *Retroviridae* family provides ample flavors of IN. Although overall sequence identity is low, structural homology is very strong among these orthologous proteins, allowing their use as a proxy for HIV IN [17]. One of these proteins, the prototype foamy virus (PFV, genus *Spumavirinae*) IN, was ultimately crystallized as a functional intasome assembly with vDNA ends [17]. These complexes were further characterized by inclusion of tDNA and/or inhibitors [18]. The structures unveiled the intimate workings of the integration machinery and represent landmark advances in the field of retrovirology [10, 19]. The intasome consists of a dimer of IN dimers, in which the two inner subunits bridge both dimers and at the same time establish extensive contacts with the vDNA (Fig. 3c) [17]. The unprocessed vDNA ends are partially unwound inside the complex and the scissile phosphodiester bond linking the invariant 3′ CA dinucleotide to the terminal GT is coordinated by Mg^{2+} ion B (Fig. 4a). Activation and alignment of an H_2O nucleophile through coordination by the neighboring Mg^{2+} ion A results in S_N2 nucleophilic substitution on the phosphodiester with the vDNA 3′ hydroxyl as a leaving group [19]. This 3′-processed intasome can accommodate tDNA (forming a target-capture complex) in a groove between the inner monomers, where it is heavily bent (Fig. 3c) [18, 19]. The major groove is pried open, positioning the tDNA scissile phosphodiester bonds in the coordination sphere of Mg^{2+} A (Fig. 4b) [18, 19]. As the vDNA 3′ hydroxyl is still coordinated to Mg^{2+} ion B, this results in catalysis of a second S_N2 reaction where the 3′ hydroxyl attacks the tDNA phosphodiester and the tDNA 5′ of the scissile bond is released [19]. The newly formed phosphodiester is displaced by 2.3 Å from the active site after transesterification, rendering insertion of the vDNA strands into the host genomic DNA scaffold essentially irreversible (Fig. 4b) [18, 19].