# Recent Advances in Combinatorial Chemistry Applied to Development of Anti-HIV Drugs

S. Pettersson[a], I. Clotet-Codina[b], J.A. Esté[b], J.I. Borrell[a] and J. Teixidó[*,a]

[a]*Grup d'Enginyeria Molecular (GEM), Institut Químic de Sarriá (IQS), Universitat Ramon Llull, Vía Augusta 390, E-08017 Barcelona, Spain*

[b]*Laboratori de Retrovirologia IrsiCaixa, Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona 08916, Spain*

**Abstract:** A compilation of combinatorial chemistry techniques applied to anti-HIV drug development is presented in this review. This synthetic strategy together with high throughput screening assays has allowed the discovery and optimization of novel lead anti-HIV compounds.

**Keywords:** Combinatorial chemistry, synthesis, anti-HIV drugs, high throughput screening.

## INTRODUCTION

The World Health Organization (WHO) estimates that about 40 million people were living with the human immunodeficiency virus (HIV) and AIDS by the end of 2004 [1].

Studies in HIV biology have provided deep knowledge of the molecular events in the HIV life cycle, which consists of several steps: viral entry [2-8], reverse transcription [9-15], integration [11,16-22], gene expression [23,24], gene assembly [25], budding [26] and maturation [27], see Fig. (**1**). These stages serve as potential targets for designing anti-HIV drugs [28].

This sequential fragmentation of the viral cycle has been used in this review for the classification of the synthesized anti-HIV libraries of compounds in accordance with the original intended target.

Current antiretroviral therapy consists of combinations of three families of compounds: reverse transcriptase inhibitors (RTI) and protease inhibitors (PI), both directed to enzymes produced by HIV, and entry inhibitors (ENI) that target the HIV envelope glycoprotein gp41 and prevent virus-cell fusion. Reverse transcriptase inhibitors (RTI) are classified in nucleoside (NRTI) and non-nucleoside (NNRTI) types. Within the group of the NRTI, the current FDA approved drugs used for anti-HIV therapy are [29]: in 1987 Zidovudine (AZT) [30,31], in 1991 Didanosine (ddl) [32,33], in 1992 Zalcitabine (ddC) [33,34], in 1994 Stavudine (d4T) [34-36], in 1995 Lamivudine (3TC) [37], in 1997 Zidovudine/Lamivudine (combines AZT and 3TC) [38], in 1998 Abacavir [39-41], in 2000 Zidovudine/Lamivudine/Abacavir (combines AZT, 3TC and Abacavir), in 2001 Tenofovir (bis-poc PMPA) [42,43], in 2003 Emtricitabine (FTC), in 2004 Abacavir/Lamivudine (combines Abacavir and 3TC) and also in 2004 Emtricitabine/Tenofovir (combines FTC and bis-poc PMPA). Within the NNRTI, the second group of reverse transcriptase inhibitors (RTI), those actually approved by the FDA are [29]: in 1996 Nevirapine (NVP) [44-46], in 1997 Delavirdine (DLV) [47,48] and in 1998 Efavirenz (EFV) [49].

In the other family of compounds used for antiretroviral therapy, the PI, the drugs approved by the FDA are [29]: in 1995 Saquinavir (SQV) [50-52], in 1996 Ritonavir (RTV) [53-55] and Indinavir (IDV) [56], in 1997 Nelfinavir (NFV) [57,58], in 1999 Amprenavir (APV) [59-61], in 2000 Lopinavir (ABT-378/r) [62], in 2003 Atazanavir (BMS-232632) and Fosamprenavir (GW433908).

No integrase inhibitors have been approved yet but there are already compounds in early human clinical trials [63].

The only approved fusion inhibitor is Enfuvirtide (Fuseon, T-20) [64], in 2003, but there are several compounds in different clinical trial stages, such as AMD070, UK,427,857 and TAK-220 [8,29].

Nevertheless, new strategies against HIV infection have been, and are still, determ inant in controlling the emergence of HIV resistance, improving the efficacy of antiretroviral treatment and expanding the arsenal necessary to combat HIV.

Combinatorial chemistry allows the obtention of a large population of molecules in a short period of time. Therefore, it has been a useful tool for the discovery and optimization of new lead compounds in medicinal chemistry research.

Obviously, the search for new potential anti-HIV drugs has taken advantage of the benefits of such combinatorial strategy [65]. Subsequently, the synthesized libraries must be examined for specific target activity in high throughput screening (HTS). These systems are designed to identify molecules with specific properties and allow the screening of thousands of compounds per day [66-68].

A compilation of the combinatorial techniques applied to the development of anti-HIV agents is presented in this review. Some references might be missed because, especially in patents, it is difficult to discern if the libraries of the synthesized compounds were really combinatorial due to the intrinsic combinatorial nature of the general formula depicted in Markush structures. In these cases the initial virtual

*Address correspondence to this author at the Grup d'Enginyeria Molecular (GEM), Institut Químic de Sarriá (IQS), Universitat Ramon Llull, Vía Augusta 390, E-08017 Barcelona, Spain; E-mail: j.teixido@iqs.url.edu

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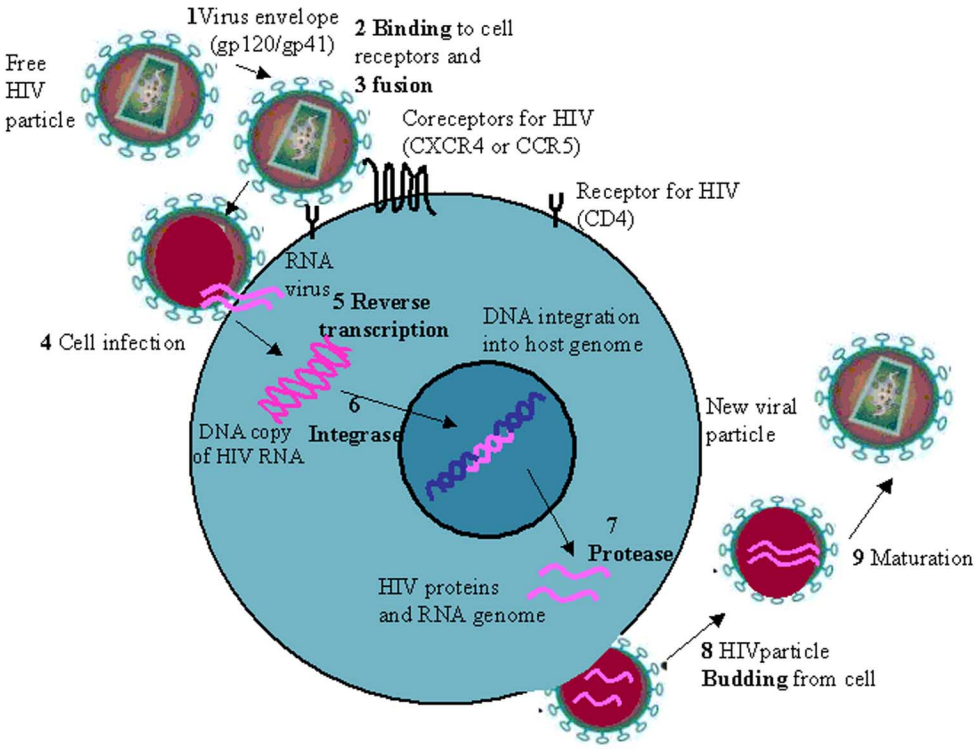



**Fig. (1).** The different steps of the HIV-1 life cycle. The text in bold refers to targets inhibited by existing agents approved by the FDA or that are likely to be inhibited in the future by new anti-HIV drugs.

library of compounds could be originally combinatorial, but a subsequent reduction of its size (i.e. using computational tools in virtual screening techniques followed by a cherry picking or sparse array selection) leads to a reduced library to be synthesized with loss of the combinatorial perception in the published work [69].

## VIRAL ENTRY

Nucleoside phosphorothioates are isoelectronic analogs of natural nucleotides in which a non bridging oxygen atom of the phosphate group is replaced by a sulfur atom [70]. This kind of compounds have shown to block the cytopathic effect of HIV-1 in uninfected ATH8 cells. These findings suggested that nucleoside phosphorothioates and their oligonucleotide analogues may represent class of experimental chemotherapeutic agents against AIDS [71].

With this background, a library of phosphorothioate oligonucleotides that contained all possible sequences of eight nucleotides divided into 16 sets, each consisting of 4,096 sequences (4 nucleotides: A, G, C, T; 8 positions; $4^8$ = 65,536 compounds), was synthesized in 1990 in solid-phase using 3*H*-1,2-benzodithiol-3-one 1,1-dioxide as sulfur-transfer reagent. The combinatorial screening of this library identified the phosphorothioate oligonucleotide $T_2G_4T_2$ as inhibitor of HIV envelope-mediated cell fusion [72]. Nevertheless, research on this oligonucleotide family has been discontinued in early clinical phases.

Another approach was finding small molecule inhibitors that bind to the coiled-coil core of gp41 subunit of HIV

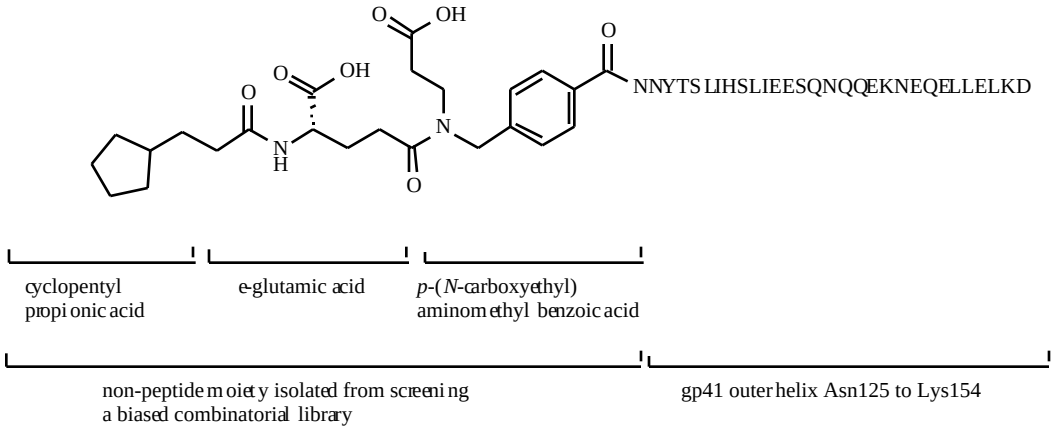



**Fig. (2).** Chemical structure of the nonpeptide moiety derived from the combinatorial library attached to the HIV-1 peptide, Asn-125 to Lys-154. The individual components from the combinatorial library are labeled in brackets.

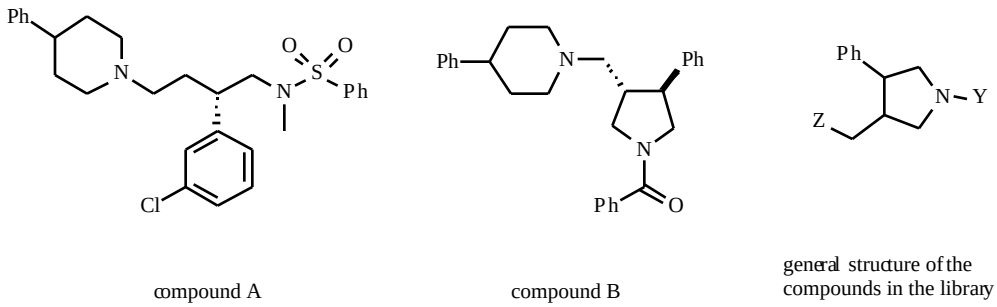



**Fig. (3).** Structure of compounds A and B and general structure of the compounds in the library generated from the SAR study around both the acyclic (A) and cyclic (B) scaffolds.

envelope and block membrane fusion, which has led to the discovery of a synthetic moiety that binds the coiled-coil when attached to the N-terminus of a 30-mer outer-layer peptide. This molecule, Fig. (**2**), was targeted by synthesizing a combinatorial library of three building blocks linked to the N-terminus of an outer-layer peptide lacking the first two α-helical turns, resulting in 61,275 of the 62,500 potential ligands from all possible combinations of 50 building blocks at the first two positions and 25 different building blocks at the third [73] (building blocks in Fig. (**4**) of the reference [74]).

A different strategy was inhibiting the binding of the virus to the CCR5 chemokine receptor. A combinatorial library was designed from a classical medicinal chemistry SAR study around compounds A and B (Fig. **3**).

The selected scaffold, see Fig. (**4**), was treated with 1,3-diisopropylcarbodimide (DIC) to form the symmetrical anhydride and then coupled to polystyrene bound arylsulfonamide in presence of DMAP. A portion of each resin was archived and the remaining resin was mixed and split into 39 equal pools. The Boc group was removed by treatment with TFA and then an acylating agent (39 different Y subunits from a variety of acid chlorides, sulfonyl chlorides and isocyanates based on the previously performed SAR study) [75-80] was added. A portion of resin from each pool was archived and the remaining resin was mixed and split into 100 equal portions. Each portion was alkylated with trimethylsilyl diazomethane followed by displacement with 2 equivalents of the amine Z subunits (selection also based on the previously performed SAR study [75-80]).

Such procedure afforded 100 pools with 117 compounds/pool. The excess amine was removed by scavenging with polystyryl isothiocyanate resin. Then, the pools were treated with borane-methyl sulfide complex to reduce the amide bonds. The desired products were obtained by treating the pools with HCl/MeOH. LC/MS analysis was performed to evaluate the composition of each pool.

The 100 mixtures were assayed for CCR5 binding affinity leading to the discovery of compound in Fig. (**5**), a potent receptor binding and with moderate anti-viral activity [81].

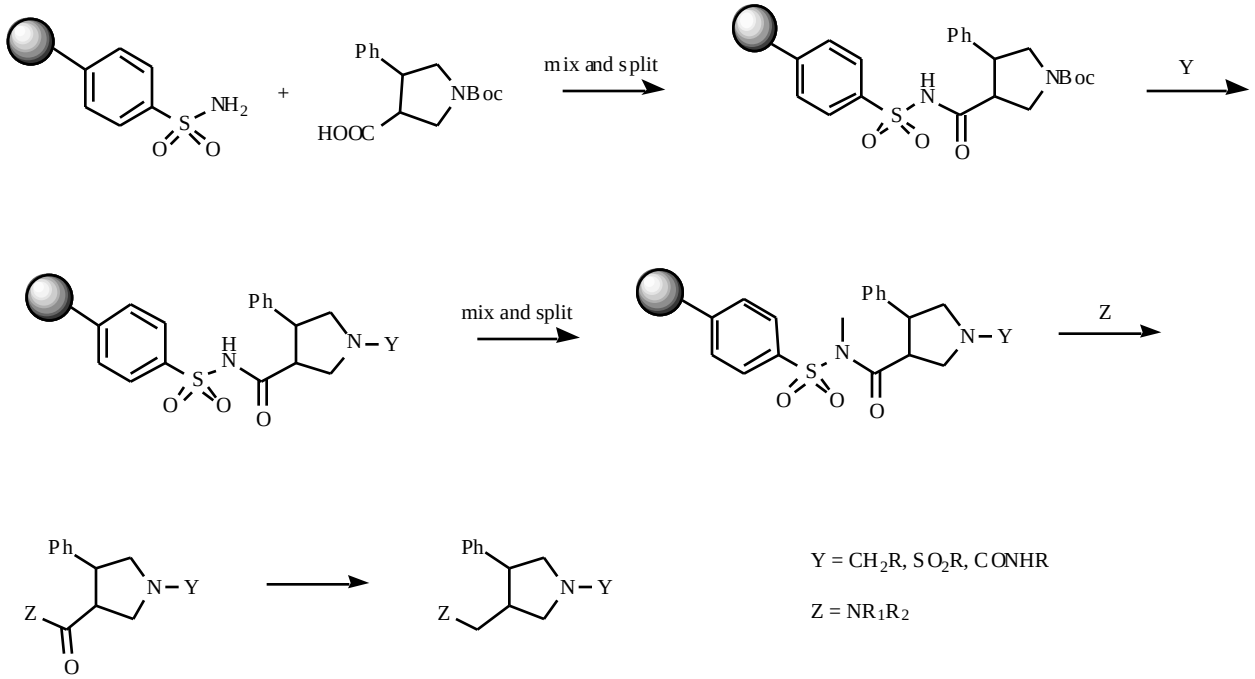



**Fig. (4).** Solid-phase synthetic route for the CCR5 antagonist library designed by Willoughby *et al.* [81] with 39 different Y subunits and 100 different Z subunits.

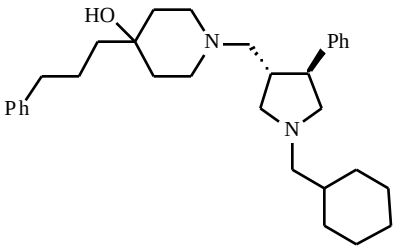



**Fig. (5).** Structure of the most potent compound in the synthesized library of CCR5 antagonists.

This compound was used for further investigations by Hale *et al*., who discovered that the incorporation of appropriate acid functional groups increased the effect on the anti HIV-1 properties of these molecules [82].

The CCR5 chemokine receptor was also used as target for a thiazolidinone library (Fig. **6**) prepared in solution phase synthesis. The first step of the synthesis was the preparation of the 4-thiazolidinone reagent pool from the corresponding arylaldehyde and 3-chloropropylamine generating an intermediate imine, which was reacted with a cyclizing agent such as mercaptoacetic acid to afford the substituted 4-thiazolidinone. These compounds were treated with NaI to afford the desired 4-thiazolidinone reagent pool, which was used for the combinatorial synthesis in array format. Each 4-thiazolidinone was treated with ten amines in separate wells and after reaction the content of each well was split into 4 new plates and treated with 4 different aldehydes [83].

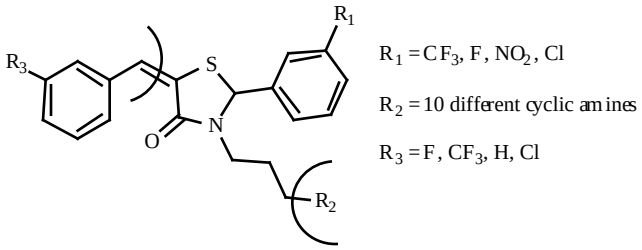



**Fig. (6).** General structure for the compounds in the library of 4-thiazolidinone. The curved lines show the different building blocks used for these structures.

Peptide libraries have been designed as possible gp120/cell membrane receptor interaction inhibitors on the basis of the crystal structure of a gp120/CD4/Fab17b complex. The synthesized libraries were H-Phe-X-X-Arg-$NH_2$ (X = Gly, Ser, Val, Phe, Lys), H-Glu-$X_1$-Glu-$X_2$-Asp-$NH_2$ (where $X_1$ = Gly, Phe, Ala, Ser, Asp, Asn and $X_2$ = Tyr, Asp, Leu, Gly, Lys, Ser; library of 36 peptides) and H-Phe-X-Arg-$NH_2$ (X = Arg, Asp, Gln, Gly, Lys, Phe, Pro, Ser, Trp, Val, None). They were prepared via both large batch and parallel split synthesis techniques.

Fmoc deprotection of the pre-swollen Rink amide resin was followed by coupling of the next amino acid using TBTU/HOBT methodology. The sequences were constructed using successive Fmoc deprotection/amino acid coupling steps. Deprotection of the final Fmoc group followed by TFA mediated cleavage from the resin and removal of acid labile side chain protecting groups afforded the desired peptides as C-terminal protected amides (Fig. **7**). The first peptide library showed a relatively good binding inhibition but no anti-viral activity. The second library was more active than the tetrapeptide library, but no clear correlation between activity and nature of the X substituent was found, and cellular assays against HIV-infected cells gave no significant activity. The third library was also not active [84].

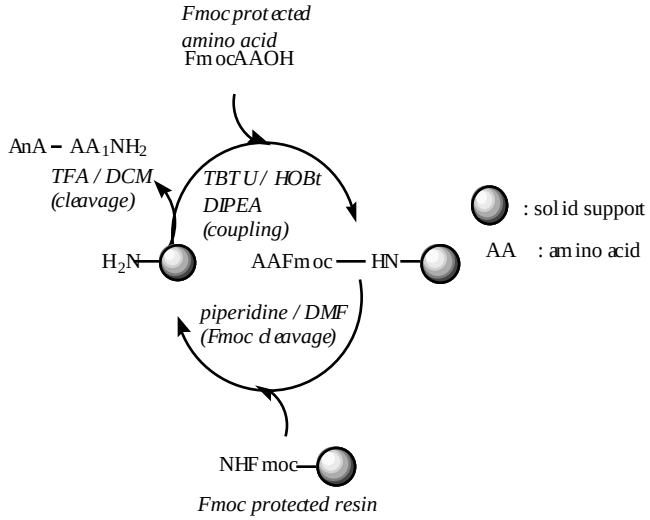



**Fig. (7).** General peptide synthesis scheme for the libraries H-Phe-X-X-Arg-$NH_2$, H-Glu-$X_1$-Glu-$X_2$-Asp-$NH_2$ and H-Phe-X-Arg-$NH_2$ reported by Boussard *et al*. [84].

## REVERSE TRANSCRIPTION

As stated in the introduction, there are two different classes of reverse transcriptase (RT) inhibitors: the nucleoside (NRTIs) and the non nucleoside (NNRTIs) inhibitors.

1-aryl-1*H*,3*H*-thiazolo[3,4-*a*]benzimidazoles (TBZs) have shown high activity as HIV-1 NNRTIs [85-87]. Structure-activity studies on these compounds suggested that the substitution on the C-1 atom of the thiazolo[3,4-*a*]benzimidazole moiety played a crucial role in the interaction of TBZs with the HIV-1 RT, especially when a 2,6-dihalo-substituted phenyl ring was present.

Therefore 2,3-diaryl-1,3-thiazolidin-4-one derivatives have been synthesized as new NNRTIs by treatment of a 2,6-

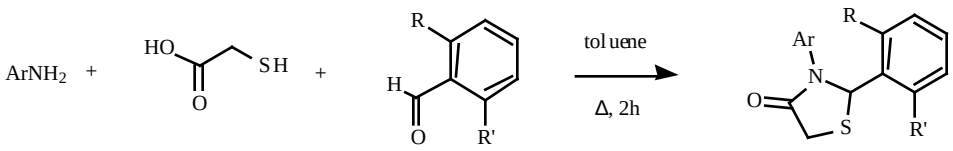



**Fig. (8).** General synthesis scheme for 2,3-diaryl-1,3-thiazolidin-4-one derivatives reported by Barreca *et al*. [88]. Where Ar = phenyl, pyridin-(2,3 or 4)-yl, 3-Me-pyridin-2-yl, 4-Me-pyridin-2-yl, 5-(Cl, Br or Me)-pyridin-2-yl, 6-(Br or Me)-pyridin-2-yl or 4,6-di-Me-pyridin-2-yl; and R,R' = Cl,Cl, F,F or Cl,F.

dihalo-substituted benzaldehyde with an equimolar amount of an (hetero)aromatic amine in the presence of an excess of mercaptoacetic acid in refluxing toluene (Fig. **8**).

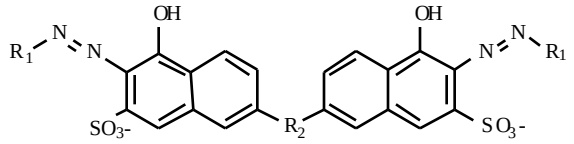


**Fig. (9).** General structures for the aryl diazo derivatives.

These compounds were up to 10-fold more potent inhibitors of replication of HIV-1 ($III_B$) or HIV-2 (ROD) in MT-4 cells than the TBZ lead compound [88].

Skillman *et al.* have used a structure-based design scheme to identify HIV-1 RT inhibitors and subsequently designed combinatorial analog libraries. A general procedure has been used for diazotization reaction and coupling to the corresponding acid.

Different aryl diazo derivatives (Fig. **9**) were synthesized with this methodology and the central urea was also replaced with a variety of linkers including thiourea, oxalyl, squarate, chelidonate, chelidamate, 2,6-pyridine dicarboxylate and terephthalate groups.

therapy (HAART) for treating neurological aspects of HIV infection [91].

A library of analogs of the natural product mappicine (Fig. **11**) has been synthesized and their activity has been tested in an HIV RNase H (ribonuclease H) assay, one of the activities of the retroviral enzyme RT.

The synthesis was performed using parallel techniques that allowed the combinatorialization of three building blocks [92].

One of the building blocks in the library, the pyridone D-ring was synthesized from a formyl pyridine where the aldehyde was reduced to a methyl group, the resulting compound was treated with *i*PrMgCl, then a TMS-iodine exchange was performed and after demethylation the iodopyridone building block was obtained.

This first building block, with associated fluorous tag, was alkylated with a propargylating agent (the second building block) and then a cascade radical annulation with the third building block, an isonitrile bearing the A-ring substituent, provided the desired mappicine analog.

This methodology was used for the synthesis of a 560-member library [93], see Fig. (**12**).

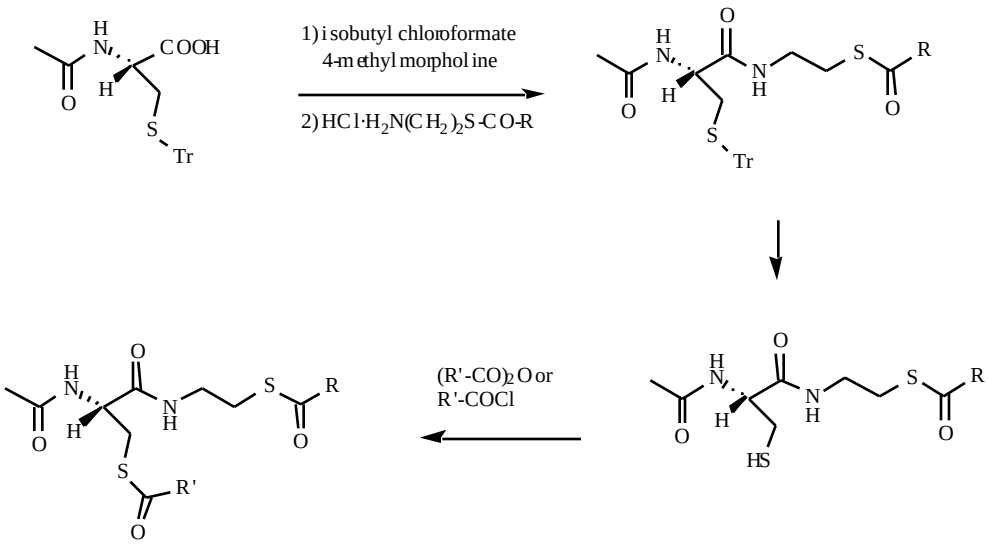



**Fig. (10).** Synthesis scheme for *N*-(*N*-acetyl-L-cysteinyl)-*S*-acetylcystemine analogs reported by Oiry *et al.* [91], where R and R' = $CH_3$, $CH(CH_3)_2$, $C(CH_3)_3$.

The most active compounds against HIV-1 RT were the urea-linked with acidic aryl diazo side-chains [89,90].

Recently a series of *N*-(*N*-acetyl-L-cysteinyl)-*S*-acetylcystemine analogs have been synthesized from commercially available *N*-acetyl-*S*-trityl-L-cysteine. The library was designed with a combinatorial approach although the synthesis was not performed with this methodology Fig. (**10**).

Compounds were tested by quantifying reverse transcriptase activity and pro-glutathione (GSH) antioxidant properties. Results showed that none of these compounds had higher values than the *N*-(*N*-acetyl-L-cysteinyl)-*S*-acetylcystemine, but they could be used as adjuvant therapies in conjunction with highly active antiretroviral

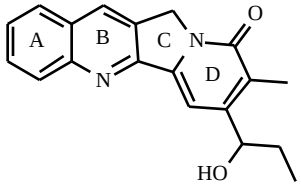


**Fig. (11).** Structure of mappicine.

## INTEGRATION

A synthetic peptide combinatorial library approach has been used for the synthesis of hexapeptides with an N-terminal free amine group and a C-terminal amide group

consisting of natural L-amino acids. This library was screened for HIV integrase inhibition in an iterative way. In the first selection step, the two N-terminal positions of the hexapeptides were defined; therefore 400 peptide mixtures were screened. Once an active peptide mixture was identified, in the second step the third position in the peptide mixtures was defined. This process continued until all positions were defined. The sequence of the most inhibiting hexapeptide was determined, His-Cys-Lys-Phe-Trp-Trp, which inhibits IN-mediated 3'-processing and integration with an $IC_{50}$ of 2 μM [94].

Structure-based computer modeling and combinatorial chemistry have been used to identify new inhibitors of HIV-1 IN, the Carbonyl J derivatives [89]; these compounds were also studied as HIV-1 RT inhibitors [90] where their structures and synthesis is detailed.

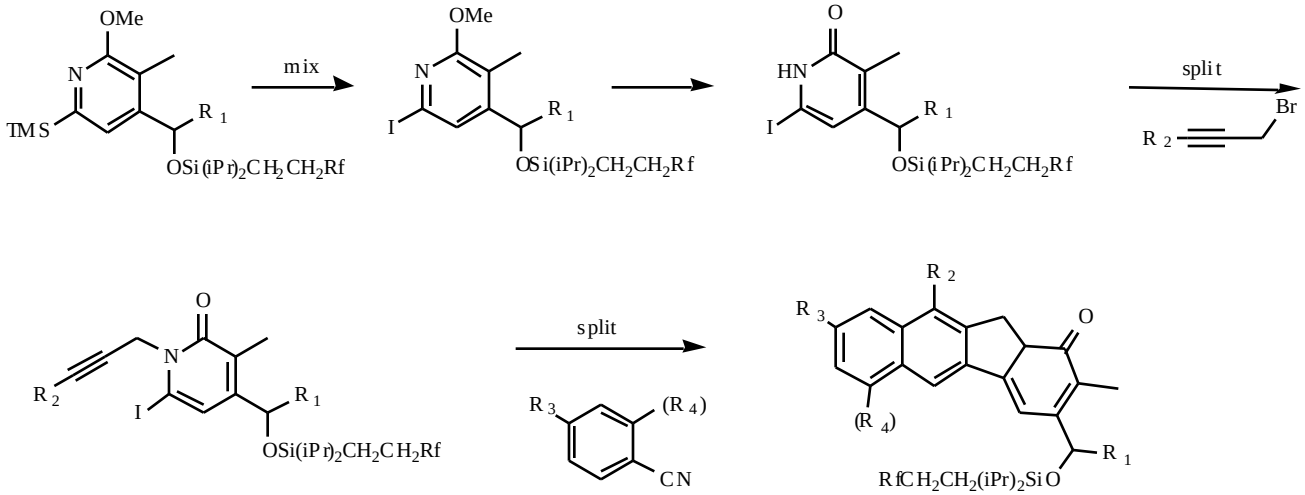
## NUCLEOCAPSID PROTEIN

Another possible target for the development of new antiviral agents is the p7 nucleocapsid protein (NCp7) of HIV-1, which is required for the functioning of the integrase enzyme as for the reverse transcriptase and protease enzymes. A series of *S*-acyl-2-mercaptobenzamide thioester derivatives were designed using a combinatorial chemistry approach. An amino acid amide was coupled with *N*,*N*'-disuccinimidyl-2,2'-dithiosalicylate, prepared from 2,2'-dithiosalicylic acid, *N*-hydroxysuccinimide and DIC. The disulfide benzamides were reduced to thiols using tris-(2-carboxyethyl)phosphine hydrochloride (TCEP·HCl) and the resulting compounds were acylated with an acid chloride to yield the desired thioesters, see Fig. (**13**).

A repertoire of 40 acyl ($R_aC$=O) groups, 6 $R_S$ substitutions on the mercaptobenzoyl substructure and 19 $NHR_L$ groups were used to render compounds with a broad anti-HIV activity. Generally good anti-HIV activity was compatible with nearly all of the acyl groups and most of the halogen substitutions on the benzoyl ring and best results were obtained with ligands that were simple amino acid primary amides as those of glycine, β-alanine, and D- or L-alanine [95].

## REV AND TAT-TAR RNA INTERACTION

A strategy for designing anti-HIV drugs is the inhibition of RNA Rev responsive element (RRE). In this context, Park *et al*. have used a one-pot Ugi-type multiple component condensation for constructing a library of




| Rf | | $R_1$ | | $R_2$ | | $R_3$ (a) | | |
|---|---|---|---|---|---|---|---|---|
| 1. $C_3F_7$ | 5. $C_8F_{17}$ | 1. Me | 5. i-Pr | 1. H | 5. Pr | 1. H | 5. p-Et | 9. p-Me |
| 2. $C_4F_9$ | 6. $C_9F_{19}$ | 2. Pr | 6. c-$C_6H_{11}$ | 2. m-MeOPh | 6. Bu | 2. p-F | 6. p-Cl | 10. p-SMe |
| 3. $C_6F_{15}$ | 7. $C_{10}F_{21}$ | 3. Et | 7. $C_2H_4$-c-$C_6H_{11}$ | 3. Me | 7. $C_5H_{11}$ | 3. p-OMe | 7. p-$OCF_3$ | |
| 4. $C_7F_{15}$ | | 4. s-Bu | | 4. Et | 8. Ph | 4. p-$CF_3$ | 8. o-F (b) | |

(a) for 1-7, 9, 10, $R_4$ = H

(b) for 8, $R_4$ = F and $R_3$ = H

**Fig. (12).** Synthetic scheme for the 560-membered mappicine analogs library with associated fluorous tags performed by Curran *et al.* [93].

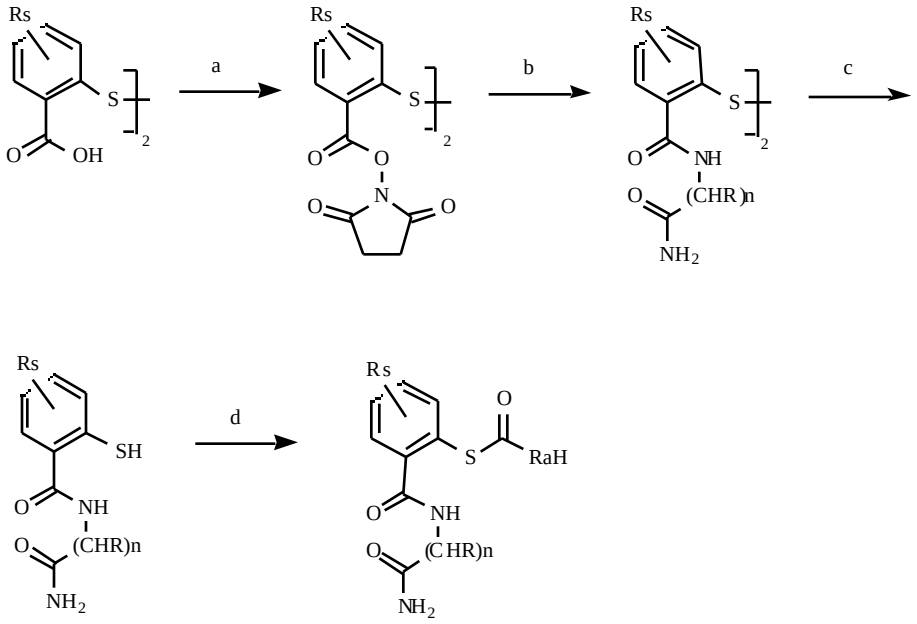



**Fig. (13).** Synthesis scheme for the 2-mercaptoamide thioesters developed by Srivastava *et al*. [95]. (a) *N*-hydroxysuccinimide/DIC/THF-*i*PrOH(7:3)/25ºC; (b) $H_2N(CHR)_nC(=O)NH_2$/DMF/25ºC; (c) TCEP·HCl/$Et_3N$/DMF-$H_2O$(9:1)/25ºC; (d) $R_aCOCl$/DMA/25ºC.

neomycin B mimetics. The starting materials were a neamine-derived aldehyde, *tert*-butyl isocyanide or isocyanoacetic acid methyl ester, a glycine-conjugated polyethylene glycol (PEG) methyl ether, and various Cbz-N-protected amino acids. Products were cleaved from PEG, hydrolyzed with basic catalysis, de-O-acetylated, and finally hydrogenated (Fig. **14** and Fig. **15**).

The peptidoaminoglycoside with X and R = H, R' = *tert*-butyl and R'' = HOOC-$CH_2$- was the most active

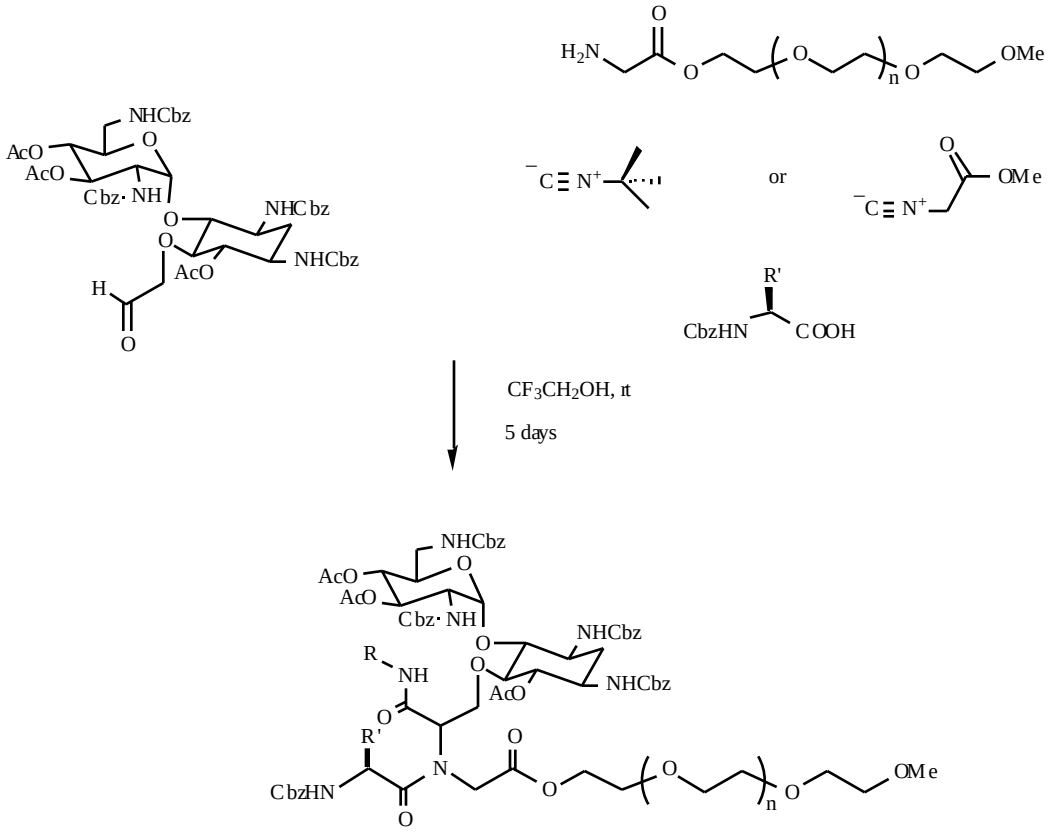



**Fig. (14).** Synthesis of neomycin B mimetics by Park *et al.* [96] using four-component condensation, where R' = Gly, Ala, Val, Phe, Trp, His, Tyr, Thr, Ser, Asp, Gln, Lys and Arg; R = *tert*-butyl, $CH_2C(O)OCH_3$ and n = ca. 113.

compound in the library and with higher activity than neomycin B [96].

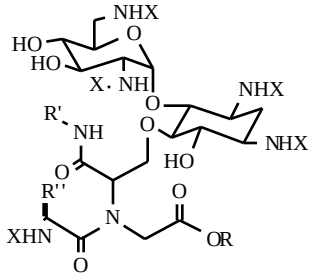


**Fig. (15).** General structure of the peptidoaminoglycosides as neomycin B mimetics, where X = Cbz and H (after removal of Cbz by hydrogenation); R = H, $CH_3$; R' = *tert*-butyl, $CH_2C(O)OCH_3$; R'' = corresponds to R' in the previous figures.

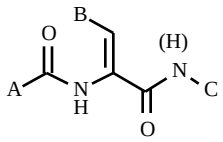


**Fig. (16).** General structure of the arylidenediamides in the library where A, B and C are different substituents (straight or branched alkyl chain, carbocyclic aryl and substituted or heterocyclic derivatives).

This strategy has also been used for the combinatorial synthesis of arylidenediamides in array format with the general structure shown in Fig. (**16**). The synthetic strategy is shown in Fig. (**17**).

An example was the synthesis of a 10,240 component array, which were synthesized from 8 oxazolones (A), 32 aldehydes (B) and 40 amines (C) [97].

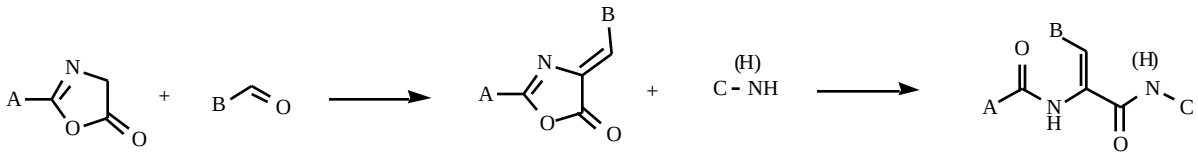


**Fig. (17).** General synthetic scheme for the compounds in the arylidenediamide library reported by Zambias *et al.* [97]. Substituents A, B and C on the molecular core are straight or branched alkyl chains, carbocyclic aryl and substituted or heterocyclic derivatives, which may also contain functional groups replacing hydrogen atoms, as tertiary amine, amide, ester, ether and halogen.

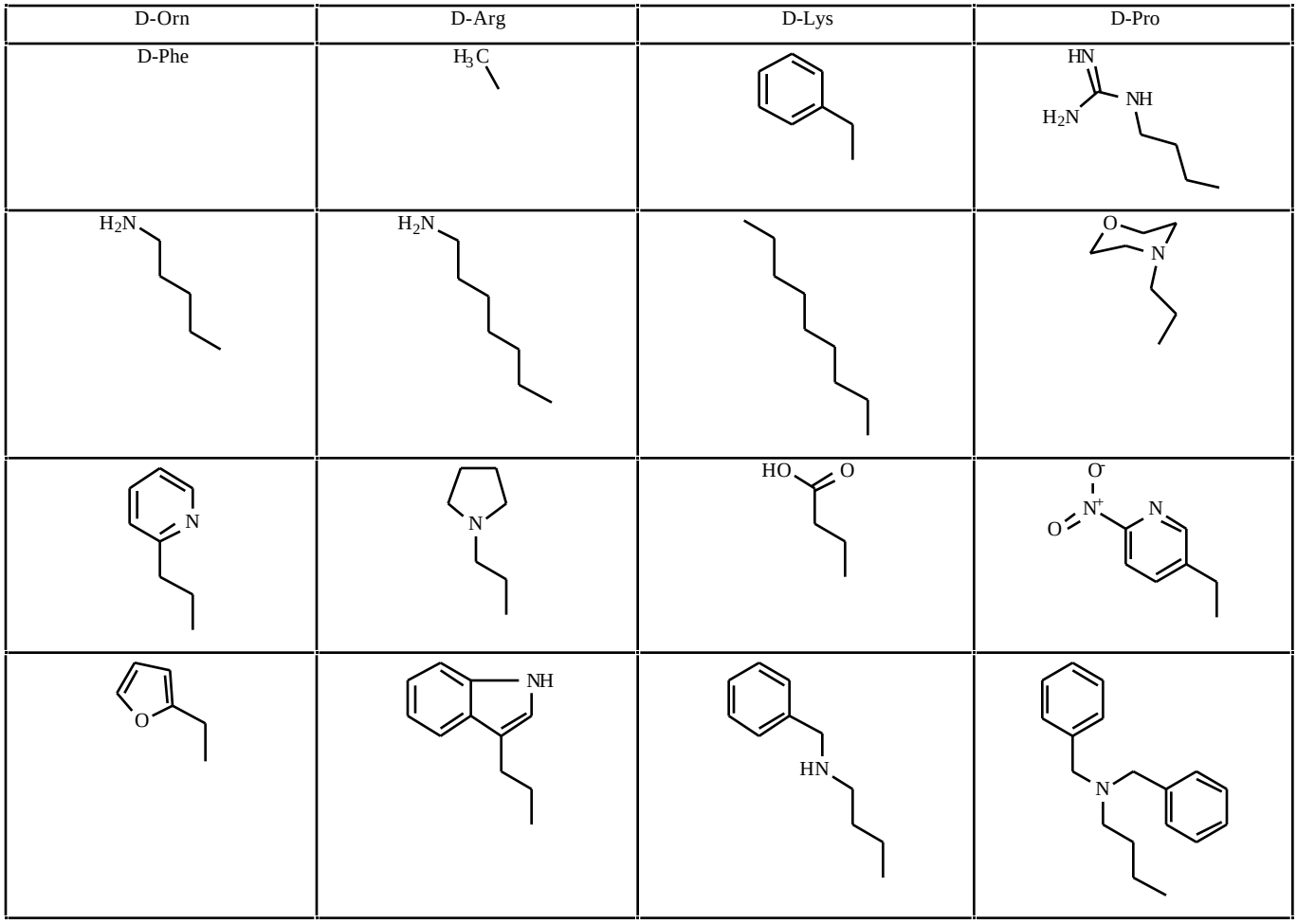



**Fig. (18).** Structure of the building blocks Hamy *et al.* [98] used to create the combinatorial peptoid library.

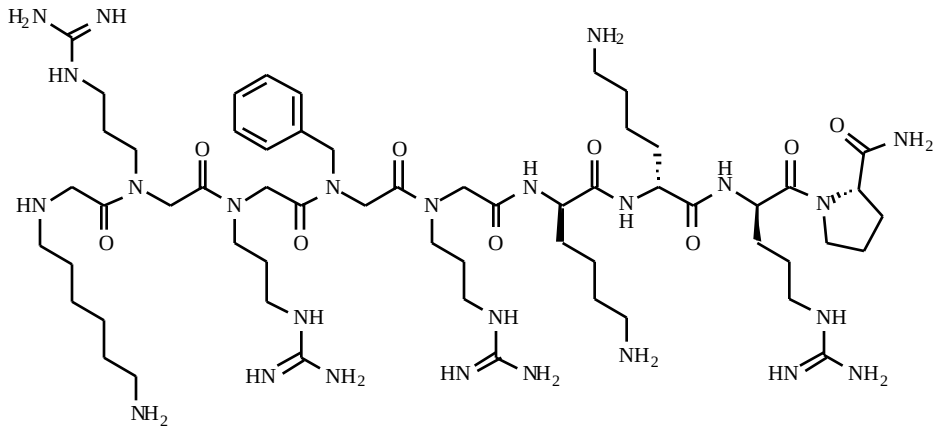


**Fig. (19).** Structure of compound CGP64222.

Another target for anti HIV-1 drugs is the inhibition of the Tat/TAR RNA interaction.

For this purpose, Hamy *et al*. synthesized a combinatorial peptoid library containing 3.2 x $10^6$ compounds divided into 20 sublibraries of 160,000 compounds each.

To limit the complexity of the library and to maintain high concentrations of individual components in the sublibraries, the four C-terminal residues were always a sequence composed of D-amino acids, D-Lys-D-Lys-D-Arg-D-Pro-amide. Five positions (residues A to E) were randomized by introducing a set of 20 building blocks carrying a wide range of functional groups (Fig. **18**).

The synthesis was accomplished by a split and mix process on a 1 % crosslinked polystyrene resin bearing the fluorenylmethoxycarbonyl-protected acid Rink amide linker.

One of the oligomers of the library specifically inhibited the Tat/TAR RNA interaction, both *in vitro* and *in vivo* (CGP64222, (Fig. **19**)) [98].

Neomycin B mimetics, arylidenediamides and the previous peptoid library have been recently proved to inhibit viral activity in the step of viral entry by acting as CXCR4 antagonist instead of the original target for which they were designed, inhibition of Tat/TAR RNA interaction [99].

An *et al*. have synthesized four unsymmetric piperazinyl polyazacyclophane scaffolds (Fig. **20**).

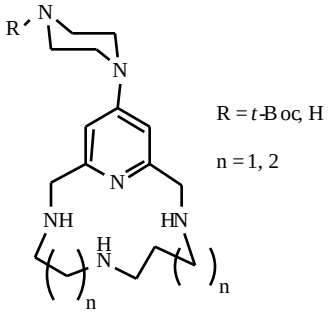



**Fig. (20).** Structure of piperazinyl polyazacyclophane scaffolds.

These scaffolds were utilized for the generation of twenty-six chemical libraries (total of 16,000 compounds) using the solution-phase simultaneous addition of functionalities (SPSAF) combinatorial approach with thirty-eight different functionalities (Fig. **21**) grouped into fourteen functionality sets A-N.

Set A included functionalities $R_1$-$R_{10}$; set B functionalities $R_{11}$-$R_{16}$; set C functionalities $R_{14}$-$R_{19}$; set D functionalities $R_{20}$-$R_{25}$; set E functionalities $R_{11}$-$R_{13}$, $R_{17}$-$R_{19}$; set F functionalities $R_{23}$, $R_{26}$-$R_{30}$; set G functionalities $R_2$, $R_8$, $R_{16}$, $R_{31}$-$R_{33}$; set H functionalities $R_{11}$-$R_{15}$, $R_{32}$; set I functionalities $R_{17}$-$R_{19}$, $R_{34}$-$R_{36}$; set J functionalities $R_{12}$, $R_{19}$, $R_{27}$, $R_{29}$, $R_{30}$, $R_{37}$; set K functionalities $R_{11}$-$R_{13}$, $R_{15}$, $R_{32}$; set L functionalities $R_9$, $R_{17}$-$R_{19}$, $R_{33}$; set M functionalities $R_{12}$, $R_{26}$, $R_{27}$, $R_{29}$, $R_{30}$; and set N functionalities $R_{12}$, $R_{19}$, $R_{28}$, $R_{29}$, $R_{38}$.

The combinatorial library was obtained by substitution of the H atoms in the NH groups of the piperazinyl polyazacyclophane scaffolds by the previously defined sets of functionalities.

These libraries were tested in HIV-1 tat/TAR protein-RNA disrupting assay using high throughput screening. Guanidine libraries 32 and 34 in the reference (with the piperazinyl polyazacyclophane scaffold with n = 1 and N substituted with set K and set M respectively) were potent inhibitors of HIV-1 tat/TAR protein-RNA interaction [100].

Recently a combinatorial library of 39,304 unnatural small molecules has been synthesized in solid phase using a set of 34 monomers and three consecutive cycles of split and pool. Monomers were selected to cover a broad range of the chemical space by varying charge, aromaticity, hydrogen bonding potential, flexibility, size, length of side chain and hydrophobicity.

The library had the general structure $NH_2$-M3-M2-M1-$NH(CH_2)_2$-O-TentaGel and was synthesized using standard Fmoc solid-phase synthetic methods, and encoded using 18 photocleavable tags. TR87 (Fig. **22**) was identified as Tat-TAR inhibitor [101].

## PROTEASE INHIBITORS

The first HIV protease inhibitor library was published by Owens *et al*. in 1991 and they identified a potent inhibitor through the screening of tetrapeptide mixtures [102].

These mixtures were synthesized as acetylated tetrapeptide amides where only one position was unique and the remaining positions contained a mixture of different amino acids; 22 amino acids plus statine (Sta) were used.

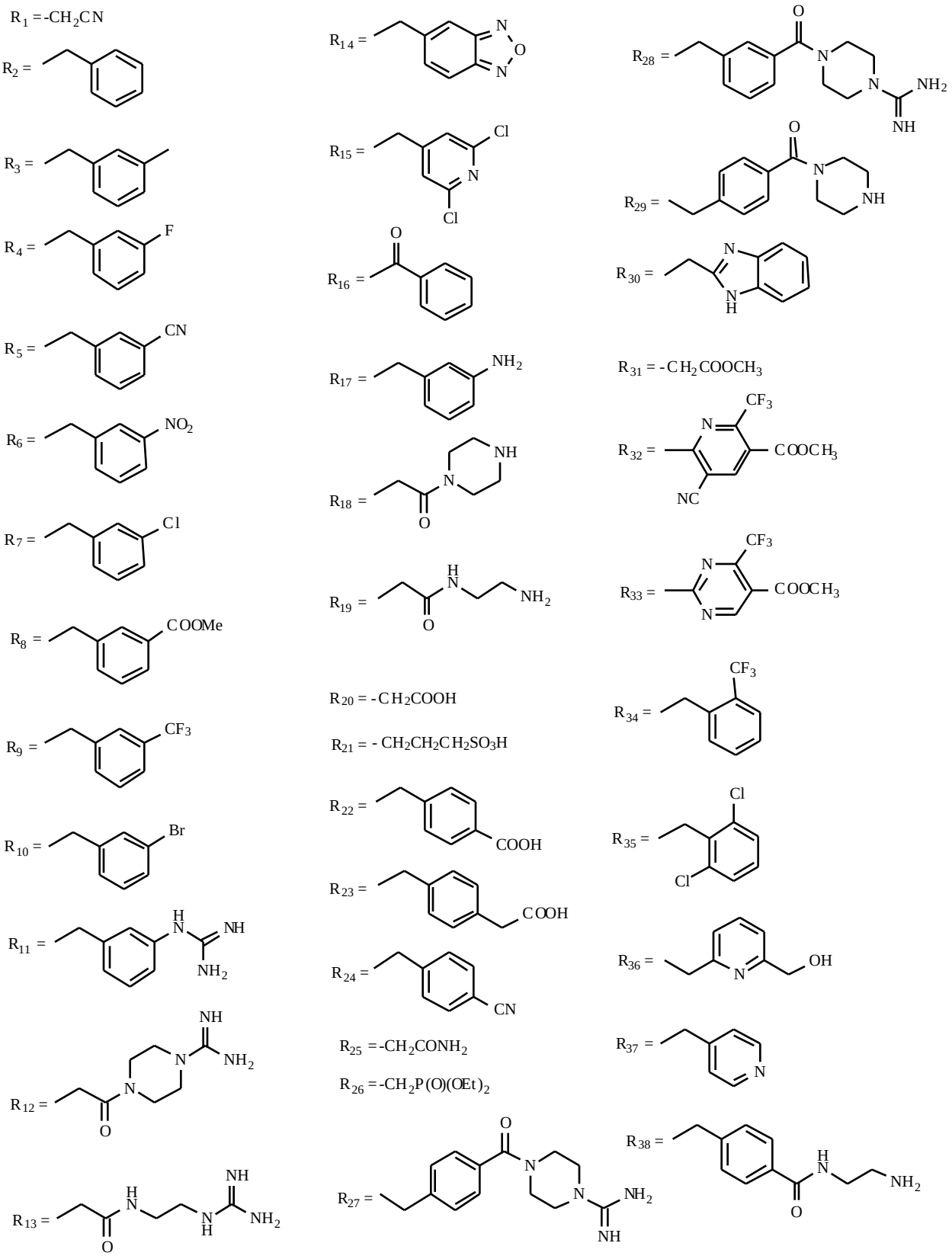



**Fig. (21).** Functionalities used for the generation of the SPSAF combinatorial library.

In the first step 22 separate Boc-amino acid-MBHA resins were prepared. The resins were combined to give Boc-$X_1$-MBHA. Then, 23 unique Boc-amino acid-$X_1$- MBHA dipeptide resins were prepared using the 22 Boc-amino acids and Boc-statine. These 23 dipeptide resins were combined to generate a new mixed resin, Boc-$Z_2$-$X_1$-MBHA, were Z is equal to X plus statine. The same procedure was used for preparing a new tripeptide resin, Boc-$X_3$-$Z_2$-$X_1$-MBHA.

Finally, 22 separate additional couplings were performed to yield 22tetrapeptide resins, Boc-$X_4$-$X_3$-$Z_2$-$X_1$-MBHA, where now the fourth $X_4$ position is defined. The peptide mixtures were cleaved from the resin with HF, and subsequently lyophilized to give 22 separate mixtures which contained 11,132 different tetrapeptide amides each.

L-ArgCar-4ABP-D-LysCar TR87

ArgCar and Lys Car: represent Arg and Lys carbamates

4ABP:

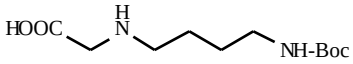



**Fig. (22).** Structure of Tat-TAR inhibitor TR87.

Positional scanning deconvolution was performed evaluating the ability to inhibit HIV protease. The most potent identified tetrapeptide was Ac-Phe-Ile-Sta-D-Leu-$NH_2$ with an $IC_{50}$ of 1.4 μM [102].

Indinavir is a protease inhibitor approved by the U.S. Federal Drug Administration as therapeutic agent for the treatment of HIV infection, whose synthesis was described in 1994 [103].

In order to improve potency and physical properties, such as short half-life and increasing viral resistance, Cheng *et al*. designed in 2000 a combinatorial library and for this purpose a solid phase synthesis route was established.

These analogues of indinavir were based on the division of the molecule into three main fragments, the aminoindanol

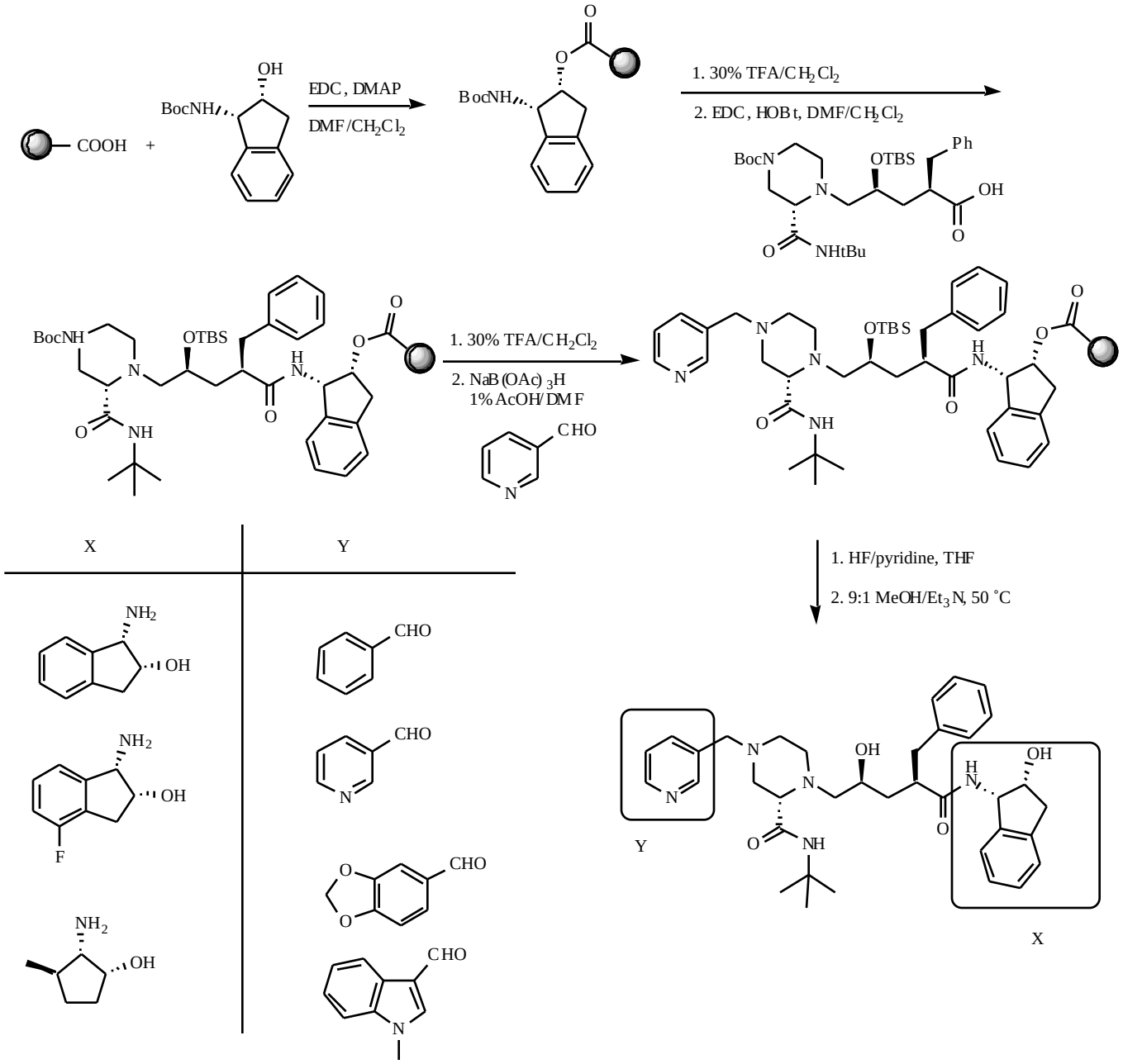



**Fig. (23).** Solid phase synthesis of indinavir reported by Cheng *et al.* [104], who follow the same methodology for the synthesis of their combinatorial library of indinavir analogs.

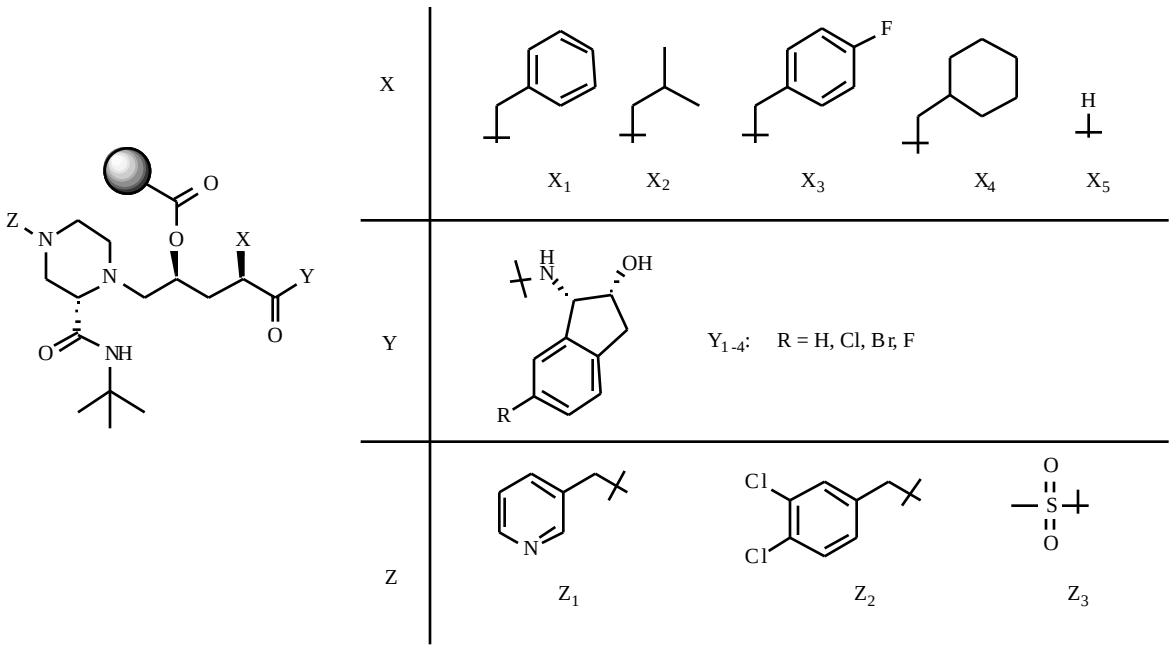



**Fig. (24).** General structure for the compounds in the library and subunits X, Y and Z synthesized by Rano *et al.* [105].

moiety, the hydroxyethylene unit and the pyridylmethyl group. The hydroxyl group on the aminoindanol was used for binding to the resin (Rapp TentaGel S COOH) *via* an ester linkage. The hydroxyethylene and pyridylmethyl fragments were coupled sequentially through amide formation and reductive amination. The final product was cleaved from the resin by transesterification with mild base (Fig. **23**) [104].

Another 'indinavir-based' combinatorial library (Fig. **24**) was designed by Rano *et al.* in order to improve the pharmacokinetic properties and *in vivo* potencies of indinavir.

A mix and split approach was used for the synthesis of these indinavir analogs diversifying the X, Y and Z subunits in (Fig. **24**). First, the resin bound hydroxyethylene isostere fragments containing 5 different X subunits were archived and mixed. The allyl group was removed and this material was split into 4 separate pools. Then, the Y subunits were attached and the pools were archived, mixed and the Boc group was removed. This resin was split into 2 separate pools followed by reductive amination with the carboxaldehyde Z subunits. Finally, the protease inhibitors were released from the resin by gentle warming in 10% TEA/MeOH. Biological activities for compounds of this library were lower than indinavir's [105].

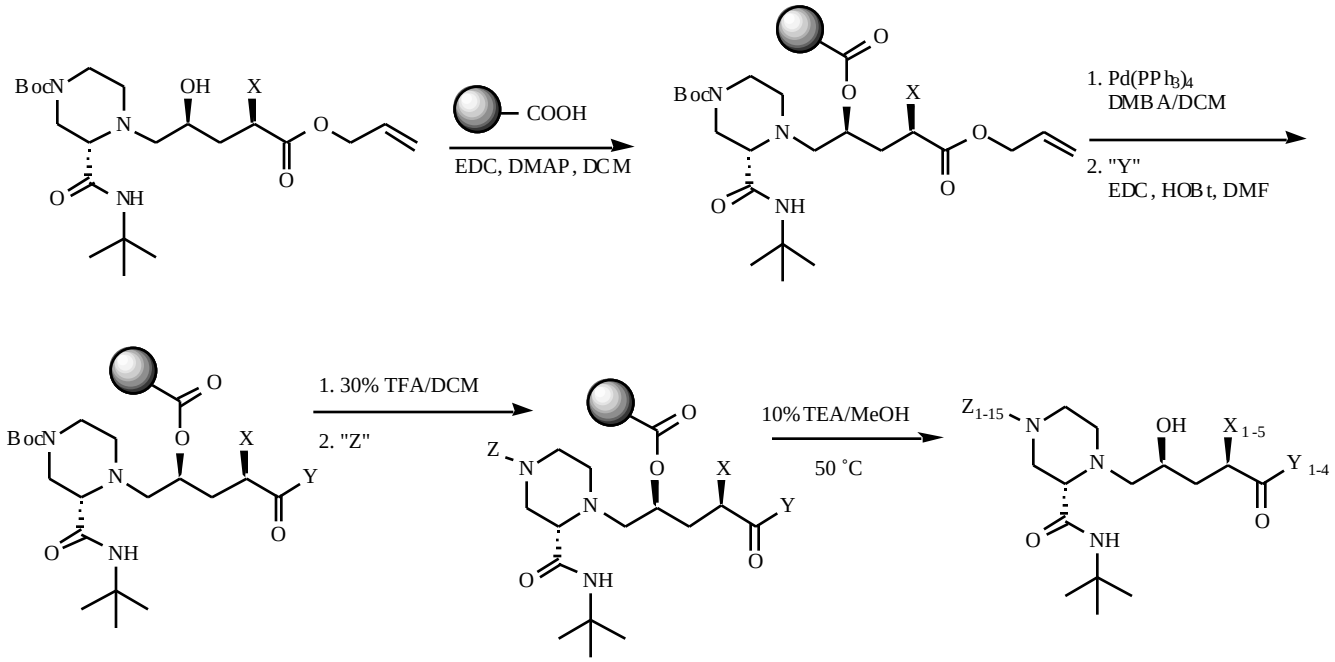



**Fig. (25).** Solid phase synthesis of the indinavir analogues library reported by Cheng *et al.* [106].

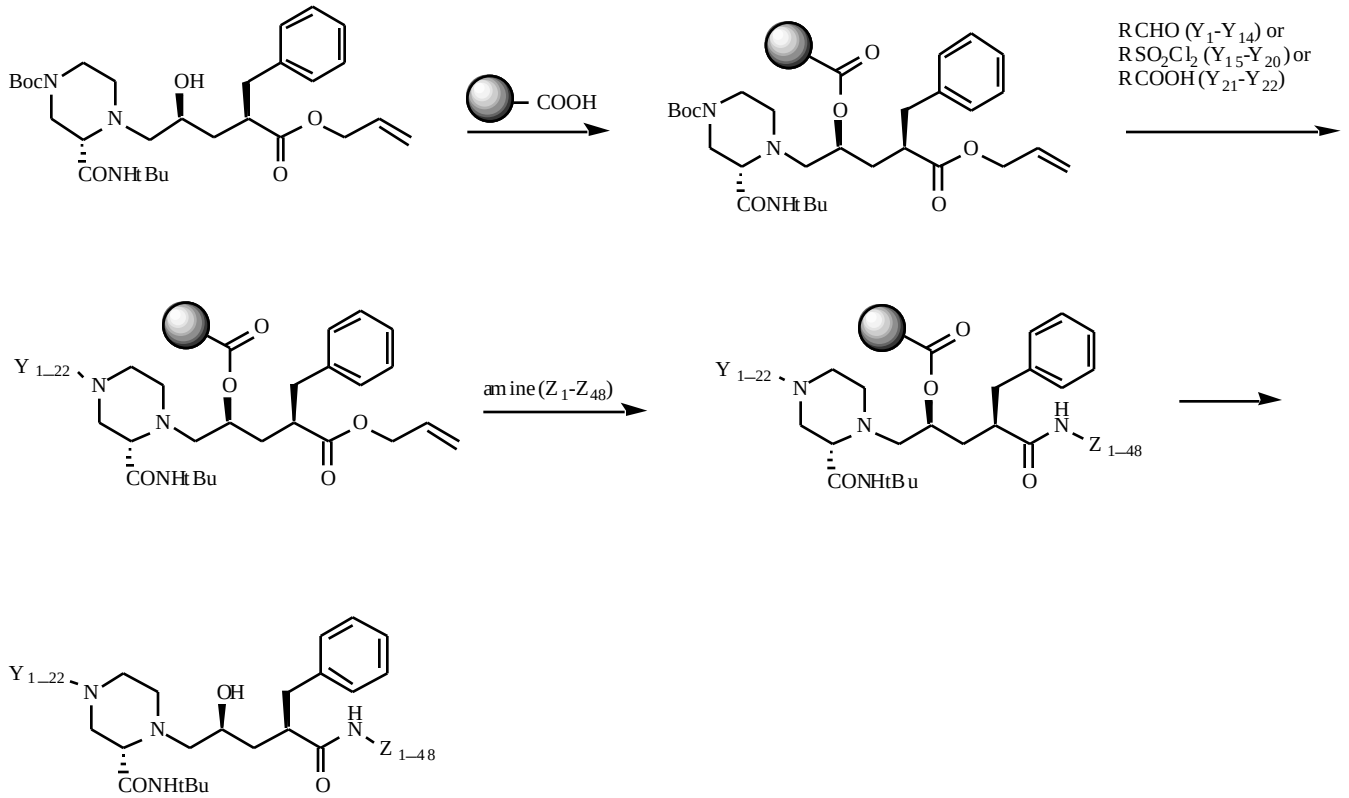



**Fig. (26).** Synthetic scheme of the library of indinavir analogues performed by Rahgavan *et al.* [107].

The aforementioned studies led to the design of another combinatorial library of indinavir analogues in 2002.

The synthesis was performed using a solid-phase mix and split protocol. The protected X subunit was anchored to the resin through the hydroxyl group via an ester linkage. Five resin bound X fragments were archived and mixed. Then, the allyl group was removed and the resulting material was split into four pools. Y subunits were attached and the four pools were archived and mixed, the Boc group being removed under acidic conditions. Then the resin was split into 15 pools and the Z subunits were attached by reductive amination, amide coupling or sulfonylation. Final products were released from the resin (Fig. **25**). The size of the library was 5 x 4 x 15 ($X_{1\text{-}5}$, $Y_{1\text{-}4}$ and $Z_{1\text{-}15}$). Their biological activity was tested as the ability to inhibit cleavage of a substrate by the wild-type HIV-1 protease enzyme, to inhibit the spread of viral infection in MT4 human T-lymphoid cells infected by the IIIb isolate and also with A-44 mutant enzyme variant PI-resistant HIV virus. Results showed that compounds X1-Y1-Z9, X1-Y1-Z10 and X1-Y1-Z15 were more potent than indinavir against both wild-type and mutant enzymes [106].

Based on the same approach, Rahgavan *et al*. synthesized a 1 x 22 x 48 combinatorial library of indinavir analogs varying the X, Y and Z subunits. The X-dimension comprised a single subunit, the hydroxyl ethylene core structure; the Y-dimension comprised 22 different subunits where aldehydes, sulfonyl chlorides and acids were included; and the Z-dimension was selected by molecular modeling with the criteria of fitting in the $S_2$ binding pocket and

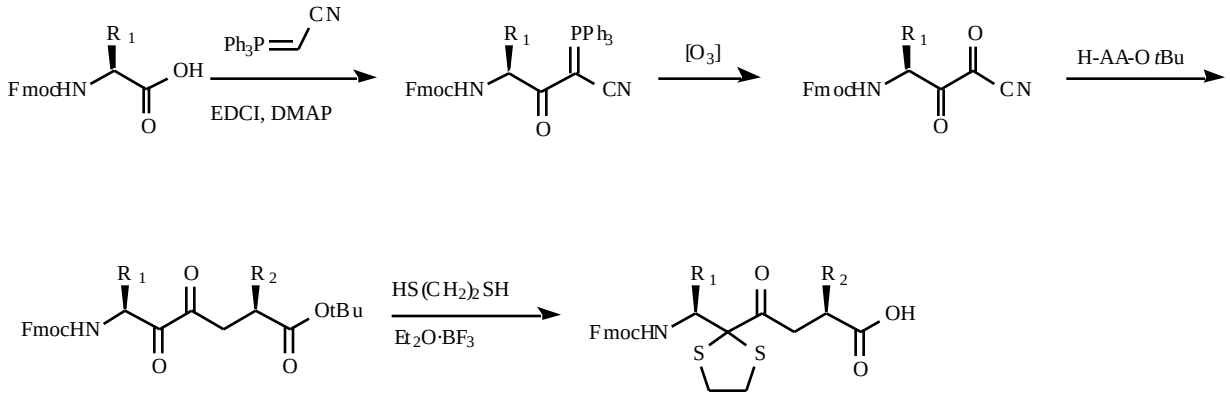



**Fig. (27).** Synthesis scheme of the α-keto amide dipeptidyl building blocks and protection of their α-keto functionality as 1,3-dithiolane with simultaneous cleavage of the *tert*-butyl ester reported by Papanikos *et al.* [109].

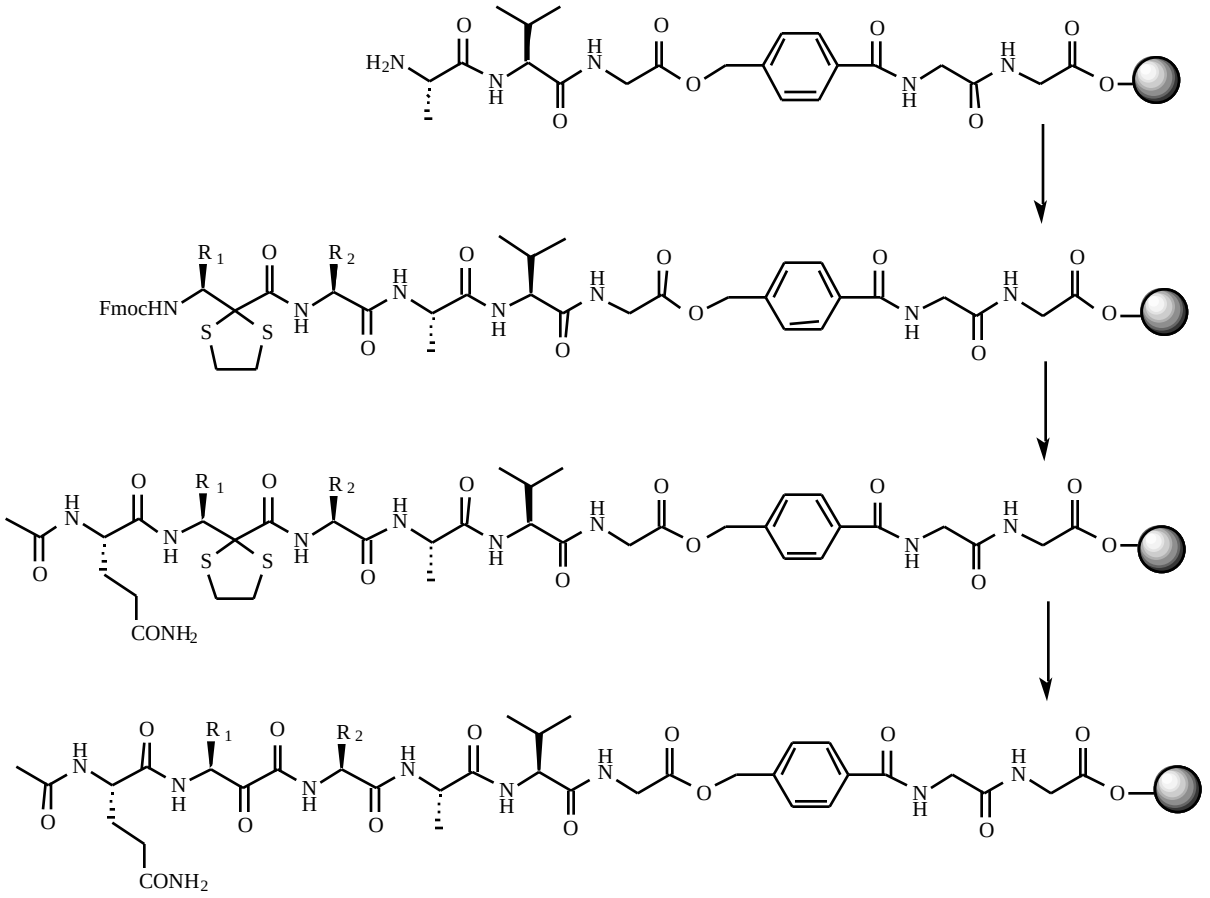


**Fig. (28).** Synthetic strategy used by Papanikos *et al.* [109] for the formation of resin-bound internal α-keto amide peptides using solid-phase peptide synthesis with the α-keto amide building blocks. $R_1$ = $CH_3$, $CH_2CH(CH_3)_2$, $CH_2Ph$ and $R_2$ = $CH_2CH(CH_3)_2$, $CH_2CH_2Ph$.

making the same kind of interactions with HIV protease enzyme as aminoindanol, therefore Z included a diverse set of aliphatic and aromatic amino alcohols and amines, some sulfones, phenols and basic amines and aminoindanol and 3-methyl-cyclopentyl amino alcohol were used as control pools. The structures of subunits X, Y and Z are shown in the reference [107].

The library was synthesized on solid support using the mix and split strategy (Fig. **26**). 2,6-dimethyl-4-hydroxy phenol was discovered to be a good replacement for aminoindanol [107].

A different strategy for HIV-1 protease inhibitors is the synthesis of α-keto amide peptides [108]. A solid-phase peptide synthetic methodology was used for this purpose. Dipeptidyl building blocks were accessible with the acylcyanophosphorane methodology and then converted into α-keto amides. Then the α-keto functionality was protected with 1,2-ethanedithiol (Fig. **27**), the resulting building blocks were assembled onto the resin to yield the desired dithiolane derivatives after Fmoc deprotection. The α-keto functionality was recovered by 1,3-dithiolane deprotection (Fig. **28**) [109].

De Michelis *et al*. have synthesized a focused library of 18 compounds incorporating the 1,3-(*N,N'*-dibenzyl)diamino-2-propanol motif based on a previous work [110] that showed that this motif elicited anti-HIV activity. The library, based on this motif, was substituted at the two end positions by various mono- or polyaminated substituted chains Fig. (**29**) [28].

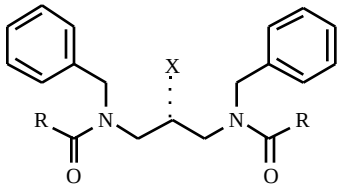


**Fig. (29).** General structure of the compounds in the library, where X = O, OH.

A different approach was the use of constrained macrocyclic templates equivalent to a tripeptide. Such templates may allow independent regioselective optimization of protease/enzyme inhibitors via focused combinatorial libraries.

The first step involved the conversion of the cyclic acid to an epoxide, which was the precursor of the desired N-terminal macrocyclic protease inhibitors, Fig (**30**). Such epoxide was regioselectively opened by primary amines to give a library of hydroxyethylamine derivatives, which were acylated by a range of sulfonyl chlorides to give a sulfonamide library or by series of isocyanates to give an urea library, see Fig. (**31**). These compounds were proved to be competitive inhibitors of HIV-1 protease [111].

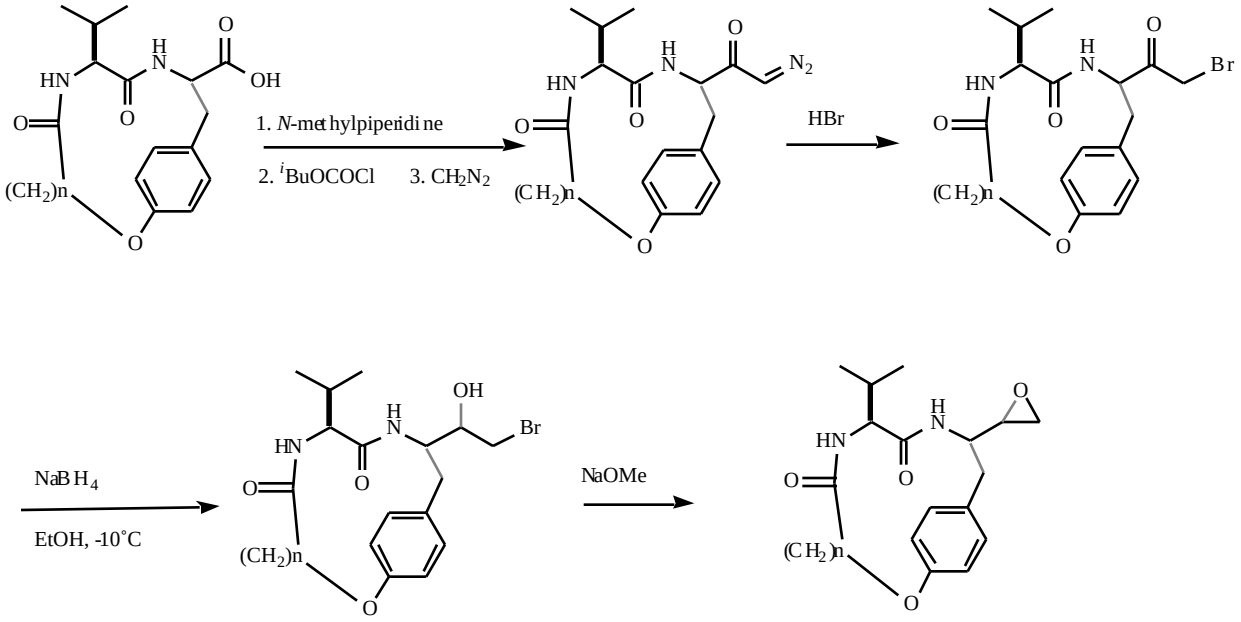



**Fig. (30).** Synthesis of the macrocyclic epoxides reported by Reid *et al.* [111].

Leroux *et al*. used a combinatorial liquid-phase strategy for the synthesis of a library of diPNA-arginine conjugates. The biological assays have not been performed, therefore their activity values, targets and modes of action have not been determined. Their design was based on the anti-HIV activity some diPNA-arginine conjugates had displayed. In the synthesis of the library diversification, see Fig. (**32**), can be achieved by varying the nature of the nucleobases $B_1$ and $B_2$ (adenine, cytosine, guanine, thymine, uracil plus the universal base analog 5-nitroindole), the length of the spacer (n) linking the arginine residue to the PNA dimer backbone (spacers composed of 2, 3, 4 and 5 methylene units were used) and, finally, the nature of *C*-extremity of arginine (carboxylic acid and methyl ester forms). Considering these variations the size of the library was $6^2$ x 4 x 2 = 288 compounds.

To synthesize this library a fully protected backbone (FPB) strategy was used. The fully protected di(aminoethylglycinamide) was selectively deprotected on the $P_1$-protected amino function, which was then stochiometrically condensed with an equimolar mixture of the six nucleobase units.

Then, the second $P_2$-amino protecting group was cleaved and another nucleobase unit was introduced. The library of diPNA-arginine conjugates was obtained by deprotection of nucleobases and the guanidinium group of arginine followed by hydrolysis [112].

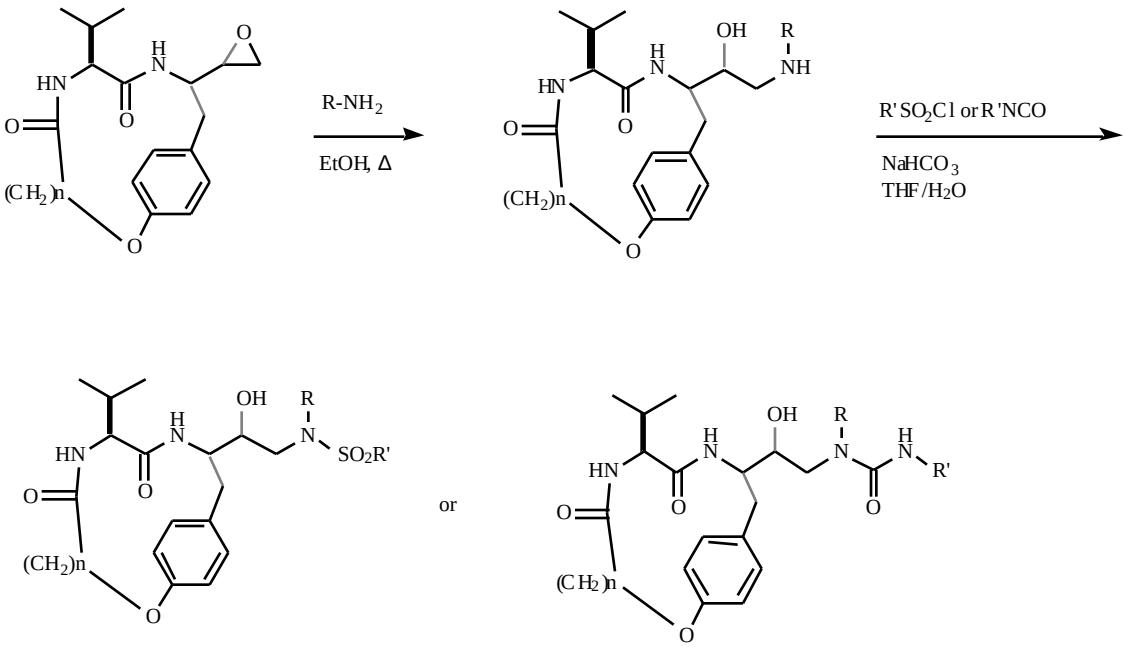



**Fig. (31).** Synthesis of N-terminal macrocyclic inhibitors developed by Reid *et al.* [111].

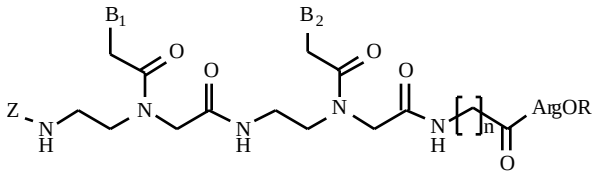



**Fig. (32).** Structure of the diPNA-arginine conjugates in the library, where $B_1$ and $B_2$ correspond to the five natural nucleobases, adenine, cytosine, guanine, thymine, uracil, and the universal base analog 5-nitroindole, n is a spacer composed of 2, 3, 4 and 5 methylenes, R is H or Me and Z represents the benzyloxycarbonyl group.

## CONCLUDING REMARKS

We have reported several combinatorial synthetic techniques, which have been applied for the discovery and generation of potential anti-HIV drugs. These synthetic techniques are mainly used in the first steps of drug discovery such as hit and lead finding. Therefore, combinatorial chemistry has proved to be a powerful tool for early stages in drug development. However, single compound syntheses are still preferred in more advanced steps although we are confident that combinatorial approach will find its place also for lead optimization purposes [69].

## ACKNOWLEDGEMENTS

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## ABBREVIATIONS

A = Adenine

Ac = Acetyl

Ala = Alanine

Arg = Arginine

Asn = Asparagine

Asp = Aspartic acid

Boc = *tert*-butoxycarbonyl

C = cytosine

Cbz = Carbobenzyloxy

Cys = Cysteine

DCM = Dichloromethane

DIC = 1,3-diisopropylcarbodimide

DIPEA = *N*,*N*-diisopropylethylamine

DMA = Dimethylacetamide

DMAP = 4-dimethylaminopyridine

DMBA = *N*,*N*-dimethylbenzylamine

DMF = *N*,*N*-dimethylformamide

EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

EDCI = 1-ethyl-3-[3-(dimethylamino)propyl]carbodi-imide

Et = Ethyl

Fmoc = Fluorenylmethoxycarbonyl

G = Guanine

Gln = Glutamine

Glu = Glutamic acid

Gly = Glycine

His = Histidine

HOBT = 1-hydroxybenzotriazole

Ile = Isoleucine

IN = Integrase

*i*Bu = Isobutyl

*i*Pr = Isopropyl

*i*PrMgCl = Isopropylmagnesium chloride

LC = Liquid chromatography

Leu = Leucine

Lys = Lysine

MBHA = 4-methylbenzhydrylamine

Me = Methyl

MS = Mass spectrometry

NRTI = Nucleoside reverse transcriptase inhibitors

NNRTI = Non-nucleoside reverse transcriptase inhibitors

Orn = Ornithine

PEG = Polyethylene glycol

Ph = Phenyl

Phe = Phenylalanine

PNA = Peptide nucleic acid

Pro = Proline

RT = Reverse transcriptase

Ser = Serine

Sta = Statine

T = Thymine

TBS = *tert*-butyldimethylsilyl

TEA = Triethanolamine

TBTU = 2-(1*H*-Benzotriazol-1-yl)-1,1,3,3-tetramethylur-onium tetrafluoroborate

*t*Bu = *tert*-butyl

TBZ = 1-aryl-1*H*,3*H*-thiazolo[3,4-a]benzimidazole

TCEP = tris-(2-carboxyethyl)phosphine

TFA = Trifluoroacetic acid

THF = Tetrahydrofuran

Thr = Threonine

TMS = Trimethylsilyl

Tr = Trityl

Trp = Tryptophan

Tyr = Tyrosine

Val = Valine

## REFERENCES


[1] http://www.who.int

[2] Maeda, K.; Nakata, H.; Ogata, H.; Koh, Y.; Miyakawa, T.; Mitsuya, H. *Curr. Opin. Pharmacol.*, **2004**, *4*, 447.

[3] Pierson, T. C.; Doms, R. W.; Poehlmann, S. *Rev. Med. Virol.*, **2004**, *14*, 255.

[4] Markovic, I.; Clouse, K. A. *Curr. HIV Res.*, **2004**, *2*, 223.

[5] Seibert, C.; Sakmar, T. P. *Curr. Pharm. Des.*, **2004**, *10*, 2041.

[6] Shaheen, F.; Collman, R. G. *Curr. Opin. Infect. Dis.*, **2004**, *17*, 7.

[7] Root, M. J.; Steger, H. K. *Curr. Pharm. Des.*, **2004**, *10*, 1805.

[8] Este, J. A. *Curr. Med. Chem.*, **2003**, *10*, 1617.

[9] Locatelli, G. A.; Cancio, R.; Spadari, S.; Maga, G. *Curr. Drug Metab.*, **2004**, *5*, 283.

[10] Milinkovic, A.; Martinez, E. *Expert Rev. Anti Infect. Ther.*, **2004**, *2*, 367.

[11] Anthony, N. J. *Curr. Top. Med. Chem.*, **2004**, *4*, 979.

[12] De Clercq, E. *Chem. Biodivers.*, **2004**, *1*, 44.

[13] Imamichi, T. *Curr. Pharm. Des.*, **2004**, *10*, 4039.

[14] Pereira, C. F.; Paridaen Judith, T. M. L. *Curr. Pharm. Des.*, **2004**, *10*, 4005.

[15] Tarby, C. M. *Curr. Top. Med. Chem.*, **2004**, *4*, 1045.

[16] Andreola, M. L. *Curr. Pharm. Des.*, **2004**, *10*, 3713.

[17] Turlure, F.; Devroe, E.; Silver, P. A.; Engelman, A. *Front. Biosci.*, **2004**, *9*, 3187.

[18] Gupta, S. P.; Nagappa, A. N. *Curr. Med. Chem.*, **2003**, *10*, 1779.

[19] Witvrouw, M.; Van Maele, B.; Vercammen, J.; Hantson, A.; Engelborghs, Y.; De Clercq, E.; Pannecouque, C.; Debyser, Z. *Curr. Drug Metab.*, **2004**, *5*, 291.

[20] Johnson, A. A.; Marchand, C.; Pommier, Y. *Curr. Top. Med. Chem.*, **2004**, *4*, 1059.

[21] Chiu, T. K.; Davies, D. R. *Curr. Top. Med. Chem.*, **2004**, *4*, 965.

[22] Parrill, A. L. *Curr. Med. Chem.*, **2003**, *10*, 1811.

[23] Devadas, K.; Dhawan, S. *Recent Research Developments in Biochemistry*, **2003**, *4*, 447.

[24] Baba, M. *Curr. Top. Med. Chem.*, **2004**, *4*, 871.

[25] Schiavoni, I.; Muratori, C.; Piacentini, V.; Giammarioli, A. M.; Federico, M. *Curr. Drug Targets Immune Endocr. Metabol. Disord.*, **2004**, *4*, 19.

[26] Morita, E.; Sundquist, W. I. *Annu. Rev. Cell Dev. Biol.*, **2004**, *20*, 395.

[27] Bukrinskaya, A. G. *Arch. Virol.*, **2004**, *149*, 1067.

[28] De Michelis, C.; Rocheblave, L.; Priem, G.; Chermann, J. C.; Kraus, J. L. *Bioorg. Med. Chem.*, **2000**, *8*, 1253.

[29] http://www.aidsinfonet.org

[30] Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; St.Clair, M. H.; Lehrman, S. N.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Broder, S. *Proc. Natl. Acad. Sci. USA*, **1985**, *82*, 7096.

[31] Herdewijn, P.; Balzarini, J.; De Clercq, E.; Pauwels, R.; Baba, M.; Broder, S.; Vanderhaeghe, H. *J. Med. Chem.*, **1987**, *30*, 1270.

[32] De Clercq, E.; Van Aerschot, A.; Herdewijn, P.; Baba, M.; Pauwels, R.; Balzarini, J. *Nucleosides Nucleotides*, **1989**, *8*, 659.

[33] Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. USA*, **1986**, *83*, 1911.

[34] Chu, C. K.; Schinazi, R. F.; Arnold, B. H.; Cannon, D. L.; Doboszewski, B.; Bhadti, V. B.; Gu, Z. P. *Biochem. Pharmacol.*, **1988**, *37*, 3543.

[35] Lin, T. S.; Schinazi, R. F.; Prusoff, W. H. *Biochem. Pharmacol.*, **1987**, *36*, 2713.

[36] Balzarini, J.; Van Aerschot, A.; Herdewijn, P.; De Clercq, E. *Biochem. Pharmacol.*, **1989**, *38*, 869.

[37] Coates, J. A.; Cammack, N.; Jenkinson, H. J.; Mutton, I. M.; Pearson, B. A.; Storer, R.; Cameron, J. M.; Penn, C. R. *Antimicrob. Agents Chemother.*, **1992**, *36*, 202.

[38] Larder, B. A.; Kemp, S. D.; Harrigan, P. R. *Science*, **1995**, *269*, 696.

[39] Daluge, S. M.; Good, S. S.; Faletto, M. B.; Miller, W. H.; St.Clair, M. H.; Boone, L. R.; Tisdale, M.; Parry, N. R.; Reardon, J. E.; Dornsife, R. E.; Averett, D. R.; Krenitsky, T. A. *Antimicrob. Agents Chemother.*, **1997**, *41*, 1082.

[40] Tisdale, M.; Alnadaf, T.; Cousens, D. *Antimicrob. Agents Chemother.*, **1997**, *41*, 1094.

[41] Faletto, M. B.; Miller, W. H.; Garvey, E. P.; St.Clair, M. H.; Daluge, S. M.; Good, S. S. *Antimicrob. Agents Chemother.*, **1997**, *41*, 1099.

[42] Arimilli, M. N.; Kim, C. U.; Dougherty, J.; Mulato, A.; Oliyai, R.; Shaw, J. P.; Cundy, K. C.; Bischofberger, N. *Antivir. Chem. Chemother.*, **1997**, *8*, 557.

[43] Robbins, B. L.; Srinivas, R., V; Kim, C.; Bischofberger, N.; Fridland, A. *Antimicrob. Agents Chemother.*, **1998**, *42*, 612.

[44] Merluzzi, V. J.; Hargrave, K. D.; Labadia, M.; Grozinger, K.; Skoog, M.; Wu, J. C.; Shih, C. K.; Eckner, K.; Hattox, S.; Adams, J. *Science*, **1990**, *250*, 1411.

[45] De Clercq, E. *Med. Res. Rev.*, **1996**, *16*, 125.

[46] Balzarini, J.; Perez-Perez, M. J.; Velazquez, S.; San Felix, A.; Camarasa, M. J.; De Clercq, E.; Karlsson, A. *Proc. Natl. Acad. Sci. USA*, **1995**, *92*, 5470.

[47] Romero, D. L.; Olmsted, R. A.; Poel, T. J.; Morge, R. A.; Biles, C.; Keiser, B. J.; Kopta, L. A.; Friis, J. M.; Hosley, J. D.; Stefanski, K. J.; Wishka, D. G.; Evans, D. B.; Morris, J.; Stehle, R. G.; Sharma, S. K.; Yagi, Y.; Voorman, R. L.; Adams, W. J.; Tarpley, W. G.; Thomas, R. C. *J. Med. Chem.*, **1996**, *39*, 3769.

[48] Romero, D. L.; Morge, R. A.; Genin, M. J.; Biles, C.; Busso, M.; Resnick, L.; Althaus, I. W.; Reusser, F.; Thomas, R. C.; Tarpley, W. G. *J. Med. Chem.*, **1993**, *36*, 1505.

[49] Young, S. D.; Britcher, S. F.; Tran, L. O.; Payne, L. S.; Lumma, W. C.; Lyle, T. A.; Huff, J. R.; Anderson, P. S.; Olsen, D. B.; Carroll, S. S. *Antimicrob. Agents Chemother.*, **1995**, *39*, 2602.

[50] Roberts, N. A.; Martin, J. A.; Kinchington, D.; Broadhurst, A., V; Craig, J. C.; Duncan, I. B.; Galpin, S. A.; Handa, B. K.; Kay, J.; Krohn, A. *Science*, **1990**, *248*, 358.

[51] Craig, J. C.; Duncan, I. B.; Hockley, D.; Grief, C.; Roberts, N. A.; Mills, J. S. *Antiviral Res.*, **1991**, *16*, 295.

[52] Ohta, Y.; Shinkai, I. *Bioorg. Med. Chem.*, **1997**, *5*, 465.

[53] Kempf, D. J.; Marsh, K. C.; Denissen, J. F.; McDonald, E.; Vasavanonda, S.; Flentge, C. A.; Green, B. E.; Fino, L.; Park, C. H. *Proc. Natl. Acad. Sci. USA*, **1995**, *92*, 2484.

[54] Markowitz, M.; Mo, H.; Kempf, D. J.; Norbeck, D. W.; Bhat, T. N.; Erickson, J. W.; Ho, D. D. *J. Virol.*, **1995**, *69*, 701.

[55] Molla, A.; Korneyeva, M.; Gao, Q.; Vasavanonda, S.; Schipper, P. J.; Mo, H. M.; Markowitz, M.; Chernyavskiy, T.; Niu, P.; Lyons, N.; Hsu, A.; Granneman, G. R.; Ho, D. D.; Boucher, C. A.; Leonard, J. M.; Norbeck, D. W.; Kempf, D. J. *Nat. Med.*, **1996**, *2*, 760.

[56] Vacca, J. P.; Dorsey, B. D.; Schleif, W. A.; Levin, R. B.; McDaniel, S. L.; Darke, P. L.; Zugay, J.; Quintero, J. C.; Blahy, O. M. *Proc. Natl. Acad. Sci. USA*, **1994**, *91*, 4096.

[57] Patick, A. K.; Mo, H.; Markowitz, M.; Appelt, K.; Wu, B.; Musick, L.; Kalish, V.; Kaldor, S.; Reich, S. *Antimicrob. Agents Chemother.*, **1996**, *40*, 292.

[58] Shetty, B. V.; Kosa, M. B.; Khalil, D. A.; Webber, S. *Antimicrob. Agents Chemother.*, **1996**, *40*, 110.

[59] Baba, M.; Okamoto, M.; Makino, M.; Kimura, Y.; Ikeuchi, T.; Sakaguchi, T.; Okamoto, T. *Antimicrob. Agents Chemother.*, **1997**, *41*, 1250.

[60] Clair, M. H. S.; Millard, J.; Rooney, J.; Tisdale, M.; Parry, N.; Sadler, B. M.; Blum, M. R.; Painter, G. *Antiviral Res.*, **1996**, *29*, 53.

[61] Kim, E. E.; Baker, C. T.; Dwyer, M. D.; Murcko, M. A.; Rao, B. G.; Tung, R. D.; Navia, M. A. *J. Am. Chem. Soc.*, **1995**, *117*, 1181.

[62] Sham, H. L.; Kempf, D. J.; Molla, A.; Marsh, K. C.; Kumar, G. N.; Chen, C. M.; Kati, W.; Stewart, K.; Lal, R.; Hsu, A.; Betebenner, D.; Korneyeva, M.; Vasavanonda, S.; McDonald, E.; Saldivar, A.; Wideburg, N.; Chen, X.; Niu, P.; Park, C.; Jayanti, V.; Grabowski, B.; Granneman, G. R.; Sun, E.; Japour, A. J.; Norbeck, D. W. *Antimicrob. Agents Chemother.*, **1998**, *42*, 3218.

[63] Fikkert, V.; Hombrouck, A.; Van Remoortel, B.; De Maeyer, M.; Pannecouque, C.; De Clercq, E.; Debyser, Z.; Witvrouw, M. *AIDS*, **2004**, *18*, 2019.

[64] Kilby, J. M.; Hopkins, S.; Venetta, T. M.; DiMassimo, B.; Cloud, G. A.; Lee, J. Y.; Alldredge, L.; Hunter, E.; Lambert, D.; Bolognesi, D.; Matthews, T.; Johnson, M. R.; Nowak, M. A.; Shaw, G. M.; Saag, M. S. *Nat. Med.*, **1998**, *4*, 1302.

[65] Maclean, D.; Martin, E. J. *J. Comb. Chem.*, **2004**, *6*, 1.

[66] Liu, S.; Jiang, S. *Curr. Pharm. Des.*, **2004**, *10*, 1827.

[67] Kuhn, P.; Wilson, K.; Patch, M. G.; Stevens, R. C. *Curr. Opin. Chem. Biol.*, **2002**, *6*, 704.
[68] Marchand, C.; Neamati, N.; Pommier, Y. *Meth. Enzymol.*, **2001**, *340*, 624.
[69] Pascual, R.; Mateu, M.; Gasteiger, J.; Borrell, J. I.; Teixidó, J. *J. Chem. Inf. Comput. Sci.*, **2003**, *43*, 199.
[70] Eckstein, F. *Angew. Chem. Int. Ed. Eng.*, **1983**, *22*, 423.
[71] Iyer, R. P.; Phillips, L. R.; Egan, W.; Regan, J. B.; Beaucage, S. L. *J. Org. Chem.*, **1990**, *55*, 4693.
[72] Wyatt, J. R.; Vickers, T. A.; Roberson, J. L.; Buckheit, R. W., Jr.; Klimkait, T.; DeBaets, E.; Davis, P. W.; Rayner, B.; Imbach, J. L.; Ecker, D. J. *Proc. Natl. Acad. Sci. USA*, **1994**, *91*, 1356.
[73] Zhou, G.; Ferrer, M.; Chopra, R.; Kapoor, T. M.; Strassmaier, T.; Weissenhorn, W.; Skehel, J. J.; Oprian, D.; Schreiber, S. L.; Harrison, S. C.; Wiley, D. C. *Bioorg. Med. Chem.*, **2000**, *8*, 2219.
[74] Ferrer, M.; Kapoor, T. M.; Strassmaier, T.; Weissenhorn, W.; Skehel, J. J.; Oprian, D.; Schreiber, S. L.; Wiley, D. C.; Harrison, S. C. *Nat. Struct. Biol.*, **1999**, *6*, 953.
[75] Dorn, C. P.; Finke, P. E.; Oates, B.; Budhu, R. J.; Mills, S. G.; MacCoss, M.; Malkowitz, L.; Springer, M. S.; Daugherty, B. L.; Gould, S. L.; DeMartino, J. A.; Siciliano, S. J.; Carella, A.; Carver, G.; Holmes, K.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Lineberger, J.; Miller, M.; Schleif, W. A.; Emini, E. A. *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 259.
[76] Finke, P. E.; Meurer, L. C.; Oates, B.; Mills, S. G.; MacCoss, M.; Malkowitz, L.; Springer, M. S.; Daugherty, B. L.; Gould, S. L.; DeMartino, J. A.; Siciliano, S. J.; Carella, A.; Carver, G.; Holmes, K.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Lineberger, J.; Miller, M.; Schleif, W. A.; Emini, E. A. *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 265.
[77] Hale, J. J.; Budhu, R. J.; Mills, S. G.; MacCoss, M.; Malkowitz, L.; Siciliano, S.; Gould, S. L.; DeMartino, J. A.; Springer, M. S. *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 1437.
[78] Finke, P. E.; Meurer, L. C.; Oates, B.; Shah, S. K.; Loebach, J. L.; Mills, S. G.; MacCoss, M.; Castonguay, L.; Malkowitz, L.; Springer, M. S.; Gould, S. L.; DeMartino, J. A. *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 2469.
[79] Finke, P. E.; Oates, B.; Mills, S. G.; MacCoss, M.; Malkowitz, L.; Springer, M. S.; Gould, S. L.; DeMartino, J. A.; Carella, A.; Carver, G.; Holmes, K.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Lineberger, J.; Miller, M.; Schleif, W. A.; Emini, E. A. *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 2475.
[80] Hale, J. J.; Budhu, R. J.; Holson, E. B.; Finke, P. E.; Oates, B.; Mills, S. G.; MacCoss, M.; Gould, S. L.; DeMartino, J. A.; Springer, M. S.; Siciliano, S.; Malkowitz, L.; Schleif, W. A.; Hazuda, D.; Miller, M.; Kessler, J.; Danzeisen, R.; Holmes, K.; Lineberger, J.; Carella, A.; Carver, G.; Emini, E. *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 2741.
[81] Willoughby, C. A.; Berk, S. C.; Rosauer, K. G.; Degrado, S.; Chapman, K. T.; Gould, S. L.; Springer, M. S.; Malkowitz, L.; Schleif, W. A.; Hazuda, D.; Miller, M.; Kessler, J.; Danzeisen, R.; Holmes, K.; Lineberger, J.; Carella, A.; Carver, G.; Emini, E. A. *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 3137.
[82] Hale, J. J.; Budhu, R. J.; Mills, S. G.; MacCoss, M.; Gould, S. L.; DeMartino, J. A.; Springer, M. S.; Siciliano, S. J.; Malkowitz, L.; Schleif, W. A.; Hazuda, D.; Miller, M.; Kessler, J.; Danzeisen, R.; Holmes, K.; Lineberger, J.; Carella, A.; Carver, G.; Emini, E. A. *Bioorg. Med. Chem. Lett.*, **2002**, *12*, 2997.
[83] Justus, S. E. A.; Lee, H. T.; Pontrello, J. K.; Roth, B. D.; Sexton, K. E.; Wilson, M. W. US Patent US 6506751, 2003. (*Chem. Abstr.* 2003, *138*, 89804)
[84] Boussard, C.; Doyle, V. E.; Mahmood, N.; Klimkait, T.; Pritchard, M.; Gilbert, I. H. *Eur. J. Med. Chem.*, **2002**, *37*, 883.
[85] Chimirri, A.; Grasso, S.; Molica, C.; Monforte, A. M.; Monforte, P.; Zappala, M.; Bruno, G.; Nicolo, F.; Witvrouw, M.; Jonckeere, H.; Balzarini, J.; De Clercq, E. *Antivir. Chem. Chemother.*, **1997**, *8*, 363.
[86] Chimirri, A.; Monforte, A. M.; Monforte, P.; Rao, A.; Zappala, M.; Bruno, G.; Nicolo, F.; Pannecouque, C.; Witvrouw, M.; De Clercq, E. *Antivir. Chem. Chemother.*, **1998**, *9*, 431.
[87] Chimirri, A.; Grasso, S.; Monforte, P.; Rao, A.; Zappala, M.; Monforte, A. M.; Pannecouque, C.; Witvrouw, M.; Balzarini, J.; De Clercq, E. *Antivir. Chem. Chemother.*, **1999**, *10*, 211.
[88] Barreca, M. L.; Balzarini, J.; Chimirri, A.; De Clercq, E.; De Luca, L.; Höltje, H. D.; Höltje, M.; Monforte, A. M.; Monforte, P.; Pannecouque, C.; Rao, A.; Zappalà, M. *J. Med. Chem.*, **2002**, *45*, 5410.
[89] Maurer, K.; Tang, A. H.; Kenyon, G. L.; Leavitt, A. D. *Bioorg. Chem.*, **2000**, *28*, 140.
[90] Skillman, A. G.; Maurer, K. W.; Roe, D. C.; Stauber, M. J.; Eargle, D.; Ewing, T. J. A.; Muscate, A.; Davioud-Charvet, E.; Medaglia, M. V.; Fisher, R. J.; Arnold, E.; Gao, H. Q.; Buckheit, R.; Boyer, P. L.; Hughes, S. H.; Kuntz, I. D.; Kenyon, G. L. *Bioorg. Chem.*, **2002**, *30*, 443.
[91] Oiry, J.; Mialocq, P.; Puy, J.-Y.; Fretier, P.; Dereuddre-Bosquet, N.; Dormont, D.; Imbach, J.-L.; Clayette, P. *J. Med. Chem.*, **2004**, *47*, 1789.
[92] de Frutos, O.; Curran, D. P. *J. Comb. Chem.*, **2000**, *2*, 639.
[93] Curran, D. P.; Parniak, M. A.; Gabarda, A.; Zhang, W.; Luo, Z.; Hiu-tung, C. C. PCT Int. Appl. WO 03074524, 2003. (*Chem. Abstr.* **2003**, *139*, 246140)
[94] Lutzke, R. A. P.; Eppens, N. A.; Weber, P. A.; Houghten, R. A.; Plasterk, R. H. A. *Proc. Natl. Acad. Sci. USA*, **1995**, *92*, 11456.
[95] Srivastava, P.; Schito, M.; Fattah, R. J.; Hara, T.; Hartman, T.; Buckheit, R. W.; Turpin, J. A.; Inman, J. K.; Appella, E. *Bioorg. Med. Chem.*, **2004**, *12*, 6437.
[96] Park, W. K. C.; Auer, M.; Jaksche, H.; Wong, C. H. *J. Am. Chem. Soc.*, **1996**, *118*, 10150.
[97] Zambias, R. A.; Bolton, D. A.; Hogan, J. C.; Furth, P.; Casebier, D.; Tu, C.; Arenas, J. E. PCT Int. Appl. WO 9635953, **1996**. (*Chem. Abstr.* **1996**, *126*, 54853)
[98] Hamy, F.; Felder, E. R.; Heizmann, G.; Lazdins, J.; Aboul-ela, F.; Varani, G.; Karn, J.; Klimkait, T. *Proc. Natl. Acad. Sci. USA*, **1997**, *94*, 3548.
[99] Cabrera, C.; Gutierrez, A.; Barretina, J.; Blanco, J.; Litovchick, A.; Lapidot, A.; Clotet, B.; Este, J. A. *Antiviral Res.*, **2002**, *53*, 1.
[100] An, H.; Haly, B. D.; Cook, P. D. *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 2345.
[101] Hwang, S.; Tamilarasu, N.; Kibler, K.; Cao, H.; Ali, A.; Ping, Y. H.; Jeang, K. T.; Rana, T. M. *J. Biol. Chem.*, **2003**, *278*, 39092.
[102] Owens, R. A.; Gesellchen, P. D.; Houchins, B. J.; DiMarchi, R. D. *Biochem. Biophys. Res. Commun.*, **1991**, *181*, 402.
[103] Dorsey, B. D.; Levin, R. B.; McDaniel, S. L.; Vacca, J. P.; Guare, J. P.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A. *J. Med. Chem.*, **1994**, *37*, 3443.
[104] Cheng, Y.; Lu, Z.; Chapman, K. T.; Tata, J. R. *J. Comb. Chem.*, **2000**, *2*, 445.
[105] Rano, T. A.; Cheng, Y.; Huening, T. T.; Zhang, F.; Schleif, W. A.; Gabryelski, L.; Olsen, D. B.; Kuo, L. C.; Lin, J. H.; Xu, X.; Olah, T. V.; McLoughlin, D. A.; King, R.; Chapman, K. T.; Tata, J. R. *Bioorg. Med. Chem. Lett.*, **2000**, *10*, 1527.
[106] Cheng, Y.; Rano, T. A.; Huening, T. T.; Zhang, F.; Lu, Z.; Schleif, W. A.; Gabryelski, L.; Olsen, D. B.; Stahlhut, M.; Kuo, L. C.; Lin, J. H.; Xu, X.; Jin, L.; Olah, T. V.; McLoughlin, D. A.; King, R. C.; Chapman, K. T.; Tata, J. R. *Bioorg. Med. Chem. Lett.*, **2002**, *12*, 529.
[107] Raghavan, S.; Yang, Z.; Mosley, R. T.; Schleif, W. A.; Gabryelski, L.; Olsen, D. B.; Stahlhut, M.; Kuo, L. C.; Emini, E. A.; Chapman, K. T.; Tata, J. R. *Bioorg. Med. Chem. Lett.*, **2002**, *12*, 2855.
[108] Sheha, M. M.; Mahfouz, N. M.; Hassan, H. Y.; Youssef, A. F.; Mimoto, T.; Kiso, Y. *Eur. J. Med. Chem.*, **2000**, *35*, 887.
[109] Papanikos, A.; Meldal, M. *J. Comb. Chem.*, **2004**, *6*, 181.
[110] Kempf, D. J.; Codacovi, L.; Wang, X. C.; Kohlbrenner, W. E.; Wideburg, N. E.; Saldivar, A.; Vasavanonda, S.; Marsh, K. C.; Bryant, P. *J. Med. Chem.*, **1993**, *36*, 320.
[111] Reid, R. C.; Pattenden, L. K.; Tyndall, J. D. A.; Martin, J. L.; Walsh, T.; Fairlie, D. P. *J. Med. Chem.*, **2004**, *47*, 1641.
[112] Leroux, M. L.; Di Giorgio, C.; Patino, N.; Condom, R. *Tetrahedron Lett.*, **2002**, *43*, 1641.

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