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Combinatorial synthesis of the peptidomimetic inhibitors of HIV 1 protease

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A cobalt-catalyzed combinatorial synthesis of various β-phenylisoserine derivatives is described. This methodology is applied to a one-pot synthesis of pyrrolidine- containing α-hydroxyamide core structure, β-phenylisoserine-valine dipeptide isostere and α-acetyl- or α-hydroxyethyl-β-amino acid derivatives.

Inhibition of human immunodeficiency virus type 1 (HIV 1) protease, an aspartyl protease responsible for viral maturation and replication, has become an attractive strategy for the design of therapeutic agents for the treatment[1, 2] of acquired immune deficiency syndrome (AIDS). Although HIV protease can cleave a number of specific peptide bonds, it is unusual in being able to cleave the Phe-Pro and Tyr-Pro sequences found in the gene products. Since the amide bonds of Pro residues are not susceptible to cleavage by mammalian endopeptidases, it provides a basis for rational design of HIV protease inhibitors selective for the viral enzymes.

The unusual cleavage of Phe-Pro and Tyr-Pro sequences in gag and gag-pol gene products by HIV 1 protease provides a basis for the rational design of inhibitors for the virus. Many research groups are actively engaged in developing a series of potent and selective HIV 1 protease inhibitors which are designed based on the transition state mimetic concept.[3] Transition state mimetic concept has been applied to the design and synthesis of non- peptidal small molecules, the *peptidomimetics*[3]. These small molecules have emerged as an efficient surrogate for an amide (Figure 1) and their incorporation in a peptide provides, improved pharmacodynamic properties such as oral bio-availability and biological half-life. Incorporation of peptidomimetic surrogates into bio-active molecules has been the focus of intensive research over the last ten years. Replacement of proteinogenic amide bonds with suitable conformationally restricted mimics has the potential to provide information regarding the biologically active conformation of peptides. Conformationally restrained surrogates have been utilized extensively in the design and the synthesis of enzyme inhibitors, often with remarkable success. Thus, recent advances have made it clear that generating libraries of small-molecule therapeutics offers greater possibilities for molecular diversity per step with reduced synthetic time period and effort as compared to linear synthesis. As a result to this there is a great demand on synthetic chemists to produce new compounds for testing. One response to this demand has been the development of techniques to greatly increase the speed and efficiency of compound synthesis. To address this demand, very powerful chemical and biological methods[4] have been developed for the generation of

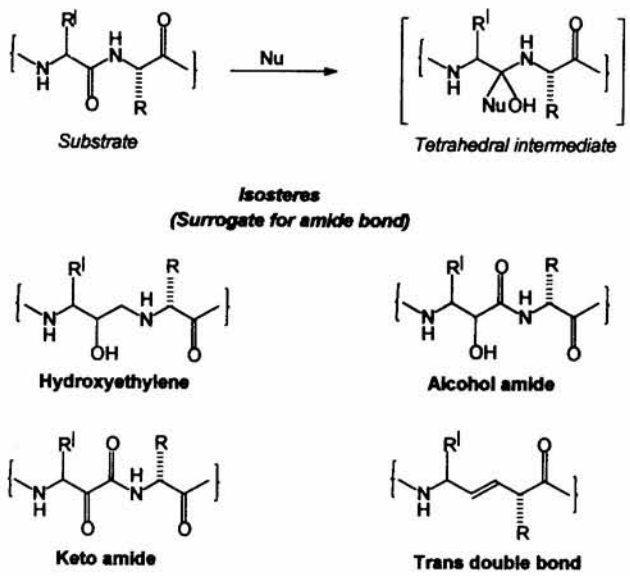


Fig. 1

large combinatorial libraries of small organic molecules. This paper describes the development of new core structures as mechanism based inhibitors of HIV-1 proteases using a combinatorial approach.

HIV proteases show high specifity for the selective cleavage of the tyrosine / phenylalanine-proline amide bonds in the matrix-capsid domain of the gag-pol polyproteins. It is this specifity that makes HIV protease an attractive target[5] for inhibition. The residues about the cleavage site (tyrosine-proline bond) at four positions P_1-P_4 and P_1'-P_4' for HIV protease is shown in Figure 2.

P_4	P_3	P_2	P_1	P_1'	P_2'	P_3'	P_4'
Ser	Gln	Asn	Tyr	Pro	Ile	Val	Gln

Figure 2

Recent studies have focussed on the development of small molecules as potential HIV protease inhibitors that span P_2- P_2' subunits in the protein. These efforts have resulted in the synthesis of mechanism-based isosteric core structures (Figure 3) such as those of hydroxyethylamine[6,7] **1**, α-ketoamide[8] **2** and phosphoric acid derivatives **3** [4].

The ongoing efforts have resulted in developing methods that could display a wide range of diverse functionality about these isosteres. Many of these inhibitors possess hydrophobic benzyl side chain (the phenylalanine side chains) at P_1 site, since large hydrophobic side chains are generally preferred at this site by the aspartic acid proteases. Recent studies have demonstrated that the introduction of additional complimentary groups in the proline ring moiety results in a significant improvement[8] in its potency. It was found that addition of a *cis*- benzyl ether to C-4 of the proline moiety increases the binding capacity threefold. The role of keto group in α- ketoamide is to enhance binding via hydration within the active site of HIV proteases. The resulting hydrate is then stabilized through hydrogen bonding interactions[9] with the aspartate residues of the enzyme. The hydrated form of α- ketoamide is considered to be a good transition state mimic based on the model shown in Figure 4.

It is amply clear from these studies that structural modification in the P_1 and P_1' subunits results in greater binding of the inhibitors with protease. It is also evident that hydrophobicity in P_1 and P_1' and aspartate assisted hydration of α-keto group play a very dominant role in enhancing this binding. Inspired by these findings, we have sought to address two main issues in our current studies. Firstly, how crucial is the presence of a phenylalanine or tyrosine residue at P_1 subunit, and secondly, can we enhance the aspartate assisted hydration by bringing the keto

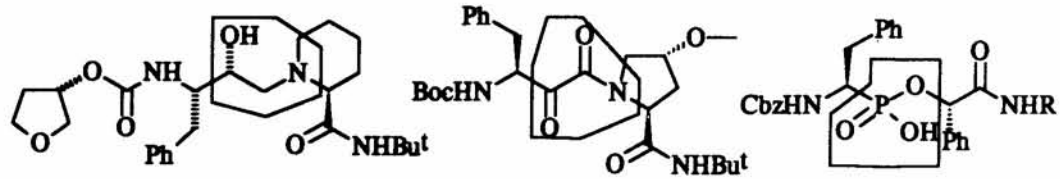


Fig. 3

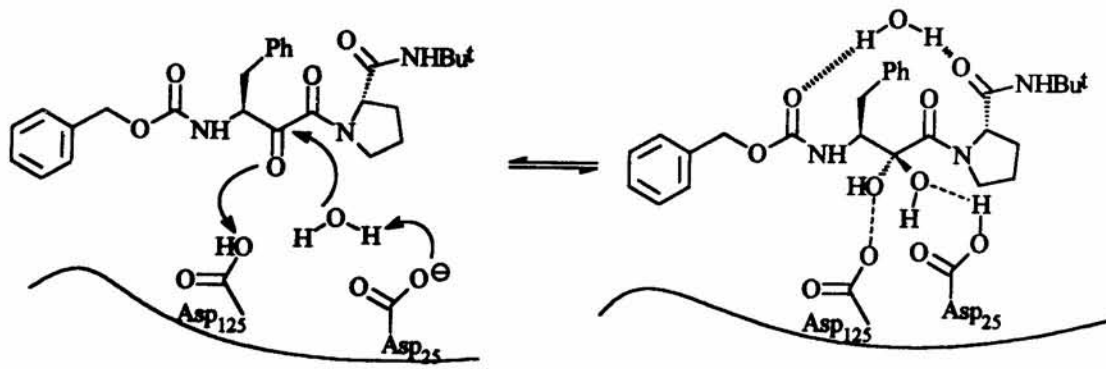
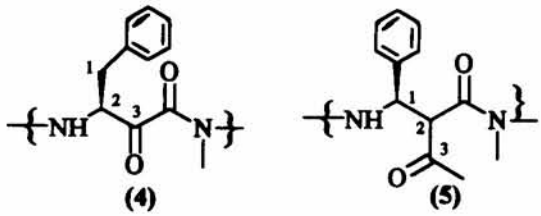
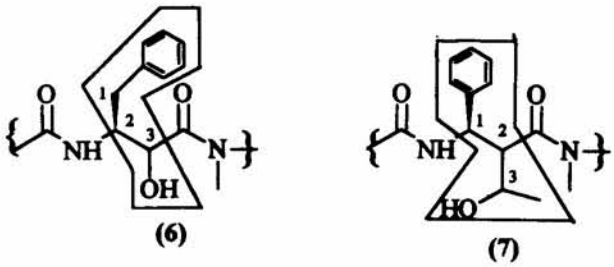


Fig. 4



(4) (5)

Fig. 5



(6) (7)

Fig. 6

group out of the main chain and place it as group in the side chain so that it can be closer to the aspartate 25 and 125. Thus, by replacing benzyl group at C-2 in **4** with phenyl group and placing an acetyl group instead of a keto at C-3 will give rise to a new structure **5** where the number of carbon atoms will not differ greatly and such an inhibitor may possess the same structural features as that of the α-ketoamide (Figure 5).

Similarly, an isosteric relationship can be established among the corresponding hydroxy compounds **6** and **7** as the resulting core structure may also have a similar isostructural relationship, where the hydroxyethyl group is placed as a branch α- to the carbonyl group (Figure 6).

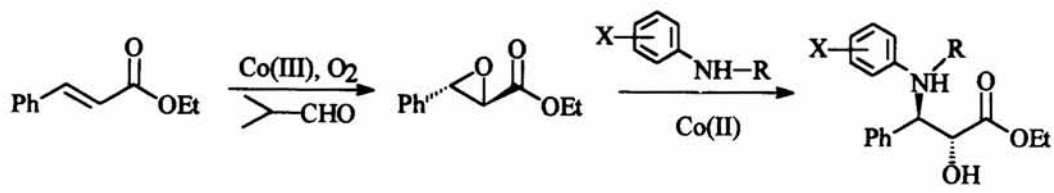
Thus, by bringing isostructural changes in the well known protease inhibitor, it is possible to create new structures which will possess the essential features of the old, one and such a modification may eventually lead to isosteres with enhanced binding properties. It is also noteworthy that the relative environment around C-2 and C-3 of **4** and **6** is not drastically changed in the modified structures **5** and **7** respectively as the overall requirement for a suitable fit within the active site of HIV protease is maintained. With this premise, we have developed a cobalt-catalyzed general route to dipeptide isosteres of β-phenylisoserine-valine, proline and β- aminoketones or esters as mechanism based inhibitors of HIV 1 proteases.

Solution Phase Strategy for the Synthesis of Chemical Libraries Containing β-Phenylisoserine-Valine and β-Phenylisoserine-Proline Dipeptide Isosters

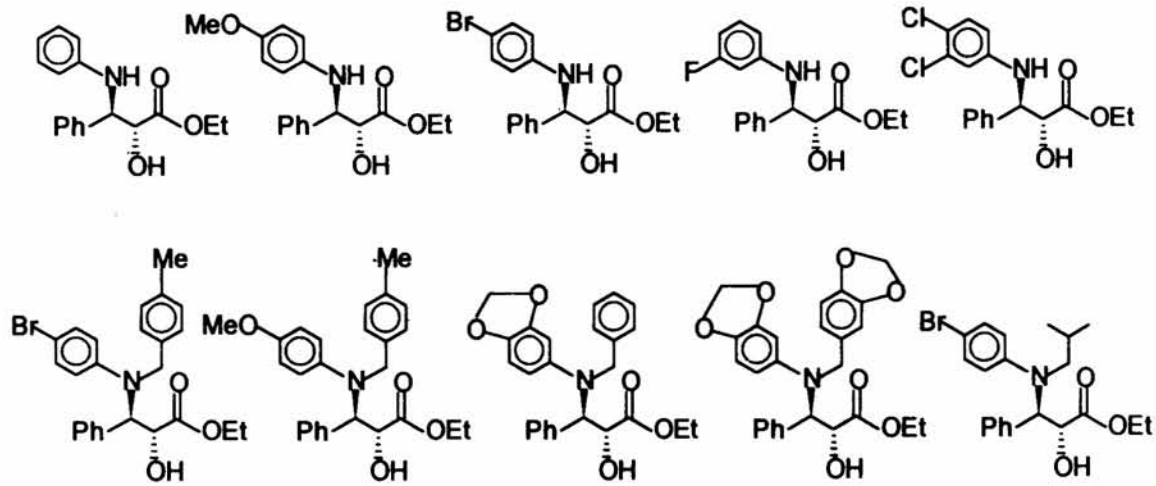
A wide range of approaches to the generation of chemical libraries have been reported[10], while solution phase synthesis has not been widely accepted as a viable alternative. Inspite of the success achieved with solid phase synthesis, there remains few bottlenecks which can be troublesome if very large number of library members are required. For example, its scale is generally restricted by the amount of required solid support and its loading capacity and the preparation of multimiligram quantaties of each library can be cumbersome and expensive. The solution phase chemistry is emerging[11] as an important complement to solid phase combinatorial synthesis mainly due to the techniques developed for the isolation of pure compounds based on liquid- liquid or solid-liquid extraction. We have persued a solution phase technique in our approach to the synthesis of dipeptide isosteres.

Earlier studies from our group have demonstrated[12] that alkenes can be epoxidized with dioxygen in the presence of a catalytic amount of cobalt complexes. We have also shown that epoxides can be cleaved[13] with anilines under the catalytic action of $CoCl_2$ at ambient conditions. We have adopted a combined application[14] of cobalt-catalyzed epoxidation followed by cleavage with anilines for the generation of functionally diverse β-phenylisoserines. Thus, treatment of ethyl cinnamate with 2- methylpropanal in acetonitrile under aerobic conditions in the presence of a catalytic amount of cobalt(III)-DMG complex at ambient conditions afforded the corresponding epoxide in good yields. This epoxide was then reacted with anilines and its derivatives in the presence of catalytic $CoCl_2$ in acetonitrile at ambient conditions to afford the corresponding *anti*-β-phenylisoserine derivatives in high yields (Scheme I).

The corresponding *syn*-diastereomers were also obtained in around 5 to 10% yields. The products were initially purified by column chromatography; however, our later attempts succeeded in isolating them by fractional crystallization. The versatility of this cobalt-catalyzed protocol was demonstrated by the preparation of a set of library containing ten distinct isoserine derivatives as shown in Scheme II.



Scheme I



α- Hydroxy - β- Amino esters

Scheme II

Our efforts on the modification of catalyst resulted in the discovery of polyaniline supported cobalt complexes which proved to be efficient[15] during epoxidation as well as cleavage with anilines. This led to the development of a one-pot procedure for the conversion of cinnamates or cinnamoyl amides to the corresponding β-phenylisoserine derivatives. The polyaniline supported cobalt complexes were prepared by mixing cobalt acetate or cobalt salen with polyemarldene base in mixed solvent system of acetonitrile / acetic acid at ambient conditions (Figure 7).

Typically, cinnamoyl amide (5 mmoles), 2-methylpropanal (15 mmoles) and (**P**)-Co(OAc)$_2$ were taken in acetonitrile and the mixture was stirred at room temperature under dioxygen balloon for 5 to 8 hr. The progress of the reaction was monitored by TLC and as soon as the starting olefin disappeared the oxygen balloon was removed and aniline (7.5 mmoles) or its derivative added, and the resulting

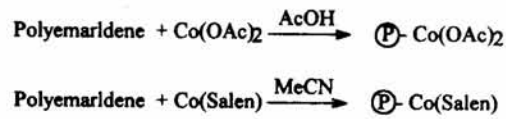
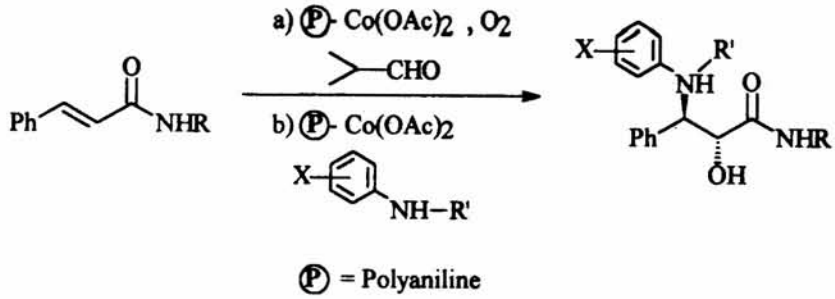


Fig. 7

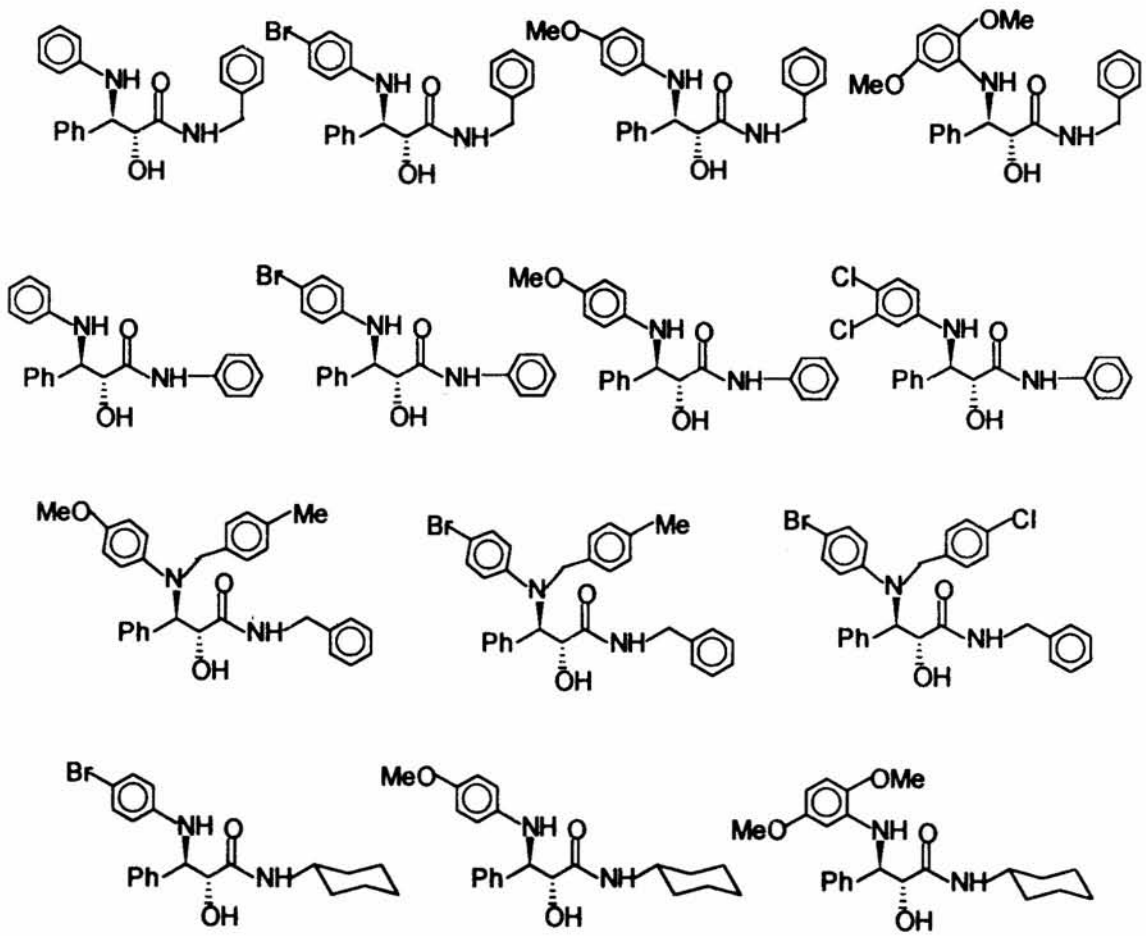
mixture stirred at ambient temperature for another 5 to 6 hr. The solvent was removed to afford a semi-solid which was washed few times with CCl_4 to afford a colourless solid in good purity (Scheme III).

This protocol circumvents the purification process by column chromatography and in most cases the purity of solid was found to be greater than 90%. This methodology was used to prepare a set of library containing fourteen distinct isoserine derivatives as shown in Scheme IV. It is interesting to note that this procedure can be used successfully to prepare dipep-



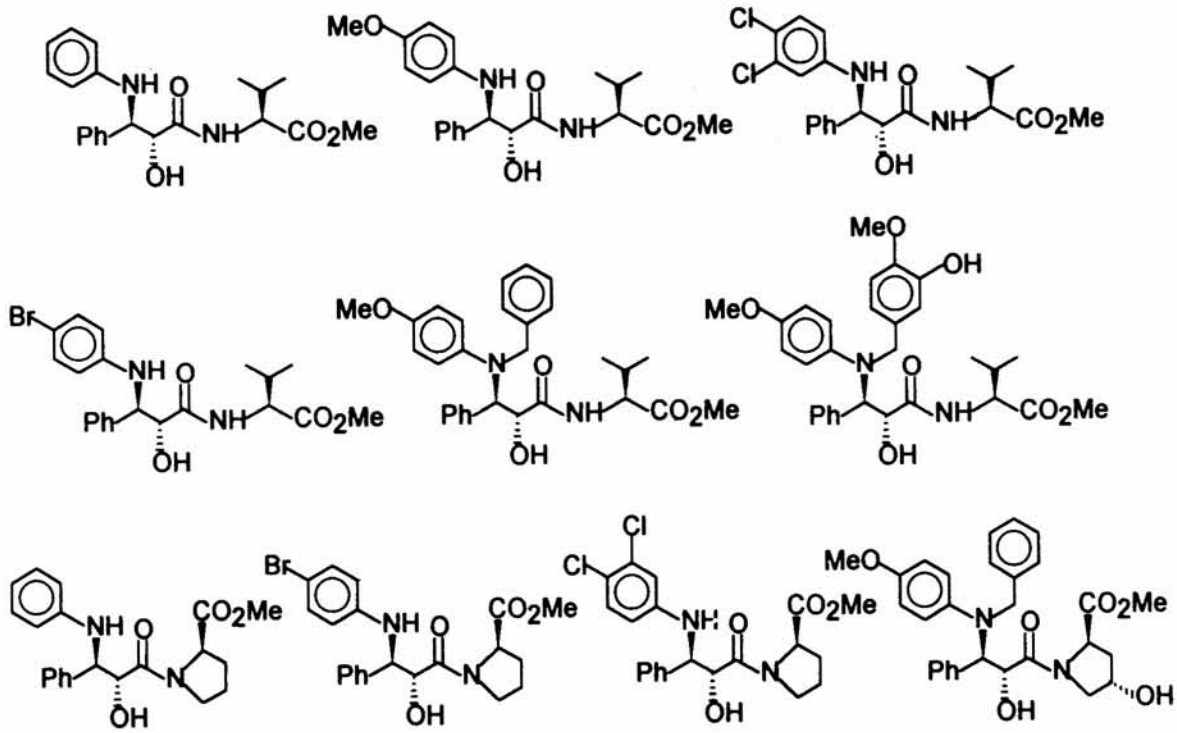
Ⓟ = Polyaniline

Scheme III



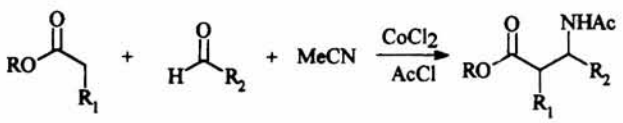
Scheme IV

tide isosteres of β-phenylisoserine and valine / proline and as per this protocol a set of ten distinct compounds were prepared from the amide derivatives of proline / valine and hydroxy proline in good yields (Scheme V). HPLC analysis of these products indicated them to be a single *anti*-diastereomers; however, the absolute configuration of the chiral centre present in them could not be ascertained. We



Dipeptide Isosteres

Scheme V



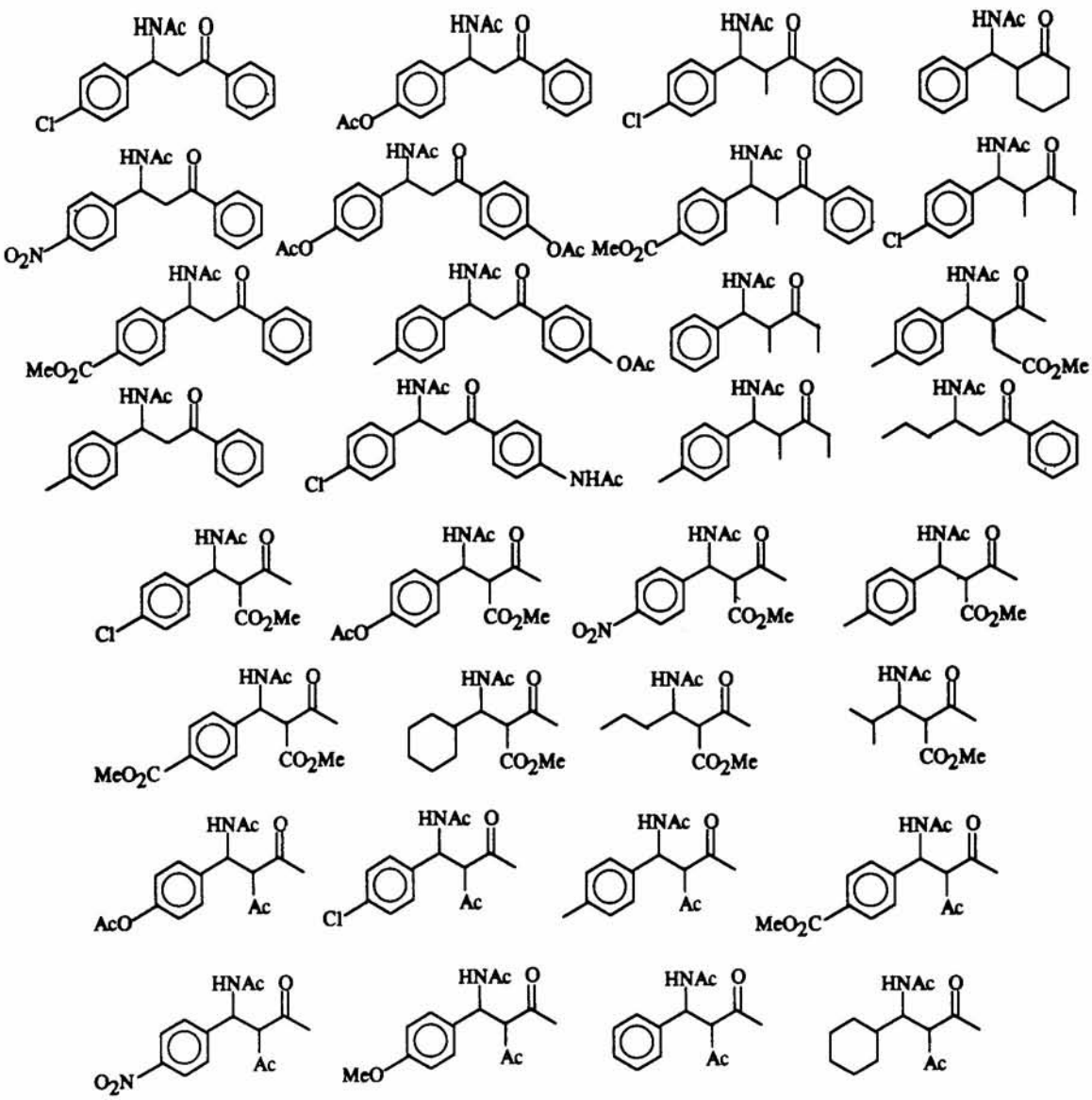
Scheme VI

are currently pursuing studies to unambiguously assign the absolute stereochemistry to these chiral centres.

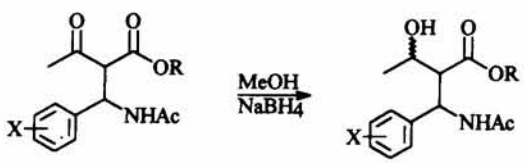
Multiple Component Condensation Protocol for the Synthesis of β-Acetamido-ketones or Esters

Multiple component condensations are those reactions in which three or more reactants come together in a single reaction vessel to form a new product which contains portions of all the components. This strategy has been used for the generation of a library of a compounds using Ugi[10] or Passerini[4] reaction. We have sought to pursue a similar approach using a cobalt-catalyzed three component coupling procedure. Our earlier studies have indicated[16] that cobalt(II) chloride catalyzes the coupling of a ketone or ketoester, aldehyde and acetonitrile in the presence of acetyl chloride to afford a high yield of β-acetamido-ketones or esters as shown in Scheme VI. A variety of ketones or ketoe-sters and aldehydes were coupled in acetonitrile to afford a diverse set of β-acetamido ketones or esters. A library of thirty two such compounds was obtained using this protocol (Scheme VII). In most of the cases the corresponding *anti*-β-acetamido-ketones were obtained as the major product.

The procedure for their isolation is very simple as most of these compounds can be isolated by passing them over a silica gel column. However, our attempts are on to purify them by a solvent extraction proce-



Scheme VII



X = NO_2, Cl, CO_2Me, Me

Scheme VIII

dure. The β-acetamido esters were reduced by $NaBH_4$ to afford the corresponding compounds (Scheme VIII) containing a hydroxyethyl side chain. These alcohols thus possess the core structure **7** (Figure 6) which can be incorporated in an amino acid residue to afford the corresponding peptide isosteres.

In conclusion, the studies described herein illustrate a highly versatile protocol for a cobalt-catalyzed three component condensation to afford a library of compounds containing β-phenylisoserine derived dipeptide isosteres and β-acetamido-ketones as potential HIV 1 protease inhibitors. The procedure for the synthesis of these compounds is very simple as in each reaction the final products were subjected to simple purification by liquid-liquid or liquid-solid extraction to remove reactants, unreacted starting materials, reagents and their byproducts providing a

library of compounds in purity irrespective of the reaction yield and without reaction optimization.

Experimental Section

General. Acetonitrile, acetic acid, carbon tetrachloride, 2- methylpropanal, olefins, different aldehydes, ketones, keto- esters, aniline and its derivatives and other solvents, reagents were used after purification according to the standard procedure. $CoCl_2$ was purchased from LOBA India Ltd., Bombay, and dried at 110°C for 2 to 3 hr before the reaction. $Co(OAc)_2$ was purchased from E. Merk, India Ltd. Co(III)DMG and Co-Salen complexes were prepared according to the literature procedure. Column chromatography was performed using 60-120 mesh (ACME) TLC silica gel or neutral alumina. ^{1}HNMR spectra were recorded at 60, 80 and 400 MHz in $CDCl_3$, DMSO-d_6 or CCl_4 using TMS as internal standard. All the starting materials were prepared according to literature procedures and compared with their ^{1}HNMR spectra. All the products were characterized by comparing with the literature data or by comparing with melting points.

General procedure for the Co-catalyzed synthesis of β-acetamido-ketones or esters. Aldehyde (10 mmoles), β-ketoester (10 mmoles) and acetyl chloride (20 mmoles) were added to a stirred solution of $CoCl_2$ (10 mg) in dry acetonitrile (50 mL). The resulting mixture was heated at 80 to 85°C for 12 to 14 hr and the progress of the reaction was monitered by TLC. The solvent was evaporated *in vacuo* and the residue taken in 50 mL of ethyl acetate. The organic layer was successively washed with $NaHCO_3$ (3×50 mL), brine (1×25 mL) and water (25mL). Drying (over $MgSO_4$) followed by evaporation of solvent gave the crude product which was purified by column chromatography (40% EtOAc in hexane).

Preparation of cobalt(II)-supported polyaniline catalyst. A mixture of polyaniline and cobalt(II) acetate (1:1, w/w) was stirred in acetonitrile and acetic acid (1:1, v/v) for 72 hr at room temperature. The reaction mixture was filtered and solid catalyst washed with acetonitrile till the filtrate became colourless. The catalyst was dried at 110 to 120°C.

Preparation of polyaniline-supported cobalt(II)-salen catalyst. A mixture of polyaniline and cobalt salen (1:1, w/w) was stirred in acetonitrile for 36 hr at room temperature. The reaction mixture was filtered and the solid catalyst washed with acetonitrile and acetic acid till the filtrate became colourless. The catalyst was dried at 110 to 120°C.

General procedure for synthesis of β-phenylisoserine derivatives.A mixture of cinnamoyl amide (5 mmoles) was dissolved in acetonitrile and 2-methylpropanal (15 mmoles) added to it, and the resulting mixture stirred in the presence of a catalytic amount of polyaniline supported cobalt(II)-salen under oxygen atmosphere at ambient temperature for 16 to 22 hr. The progress of the reaction was monitered by TLC. As soon as the starting cinnamoyl amides disappeared the oxygen balloon was removed and anilines (5 mmoles) and a small amount of catalyst were added to this reaction mixture. The mixture was stirred at 25°C for 6 to 8 hr. Removal of solvent gave a residue which was washed with CCl_4 (3×10 mL) to afford the β-phenylisoserine derivatives as crystalline solids.

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