Report

Simple Coupling Reaction between Amino Acids and Weakly Nucleophilic Heteroaromatic Amines

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Simple Coupling Reaction between Amino Acids and Weakly Nucleophilic Heteroaromatic Amines

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Introduction. The application of parallel synthesis to generate combinatorial libraries as an efficient means of creating pharmaceutical "druglike leads" has gained considerable interest.[1] By accelerating the synthesis and screening of an ever larger number of synthetic analogues, combinatorial chemistry has greatly impacted the drug discovery process.[2] Recently, we published results on combinatorial synthesis of *N*-acylthioureas[3a] and on parallel solution-phase synthesis of peptides and pseudopeptides of biological interest.[3b] Our goal was to prepare compounds that could be tested through high-throughput or medium-throughput screenings on various proteolytic enzymes involved in pathologies as different as HIV and HBV[4] or neurodegenerative disorders, such as Alzheimer's disease.[5] In our present research program on the development of new molecules as potential γ-secretase inhibitors,[6] we are interested in the solution-phase synthesis of a parallel library of new pseudopeptide derivatives bearing various weakly activated amino heteroaromatic moieties.

Our objective was to introduce through an amide bond, on biologically active peptides or pseudopeptides, substituted heterocycles, such as substituted 5-amino-tetrazoles, substituted 2-amino-thiazoles, and substituted 2-aminobenzothiazole (Figure 1), and to evaluate their anti-protease inhibitory properties. These heterocycles were selected because they provide variations in aromatic, electrostatic, and hydrogen-bonding properties: (i) all of these heterocycles can participate in hydrogen bonding as both acceptors and donors; (ii) they are aromatic systems; and (iii) they display acidic or basic properties, depending on their substituents. In addition, some of these heterocycles bearing a nitro substituent can be reduced into amine groups suitable for further coupling reactions, allowing the design of nonpeptidic receptor ligands.

At first, it seems that introduction of such heterocycles in a peptide sequence should be quite straightforward using conventional standard coupling methods, but we discovered soon that the coupling between such weakly nucleophilic heterocyclic amines and amino acids was not trifling with standard coupling reagents. At this point, we believed that N-acylation of such heterocyclic amines required preliminary investigations before extrapolation to automation on our Chemspeed automated synthesizer.

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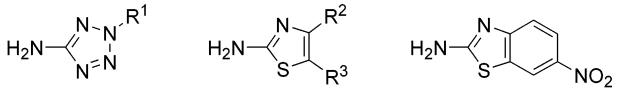

R^1 = CH_2-C_6H_5; CH_2-C_6H_4-(*p*-NO_2); CH_2-C_6H_{11}
R^2 = H; C_6H_4-(*p*-NO_2)
R^3 = H; Br; NO_2

Figure 1. Weakly nucleophilic amino heteroaromatic compound structures.

In this paper, we wish to report the results of a comparative study concerning solution-phase N-acylation between various substituted weakly nucleophilic heteroaromatic amines and a selected *N*-Boc-protected amino acid model (*N*-Boc-Ala) using different coupling reagents according to a parallel synthesis procedure. The resulting described small library of these new versatile synthons will be enlarged to a greater size by solution-phase automated synthesis and then will be used in the design of new protease inhibitors.

Results and Discussion. Preparation of amides is one of the most important and fundamental processes in organic synthesis, and many methods have been reported. Several coupling reagents of free carboxylic acids and amines have been developed in order to achieve the effective synthesis of amides.[7] However, for acylation of weakly nucleophilic amines, rather drastic reaction conditions are required for completion of the reaction. Numerous coupling reagents have been described to achieve peptide bond formation with satisfactory results (yields, mild conditions) with weakly nucleophilic amines, mainly substituted anilines, but no comparative studies have been published so far on the efficiency of standard coupling reagents to catalyze the acylation of weakly heteroaromatic amines.

For instance, acylation of anilines was achieved through the use of nonconventional coupling methods and reagents, such as silyl carboxylates,[8] trimethylsilyl polyphosphates,[9] chloranhydrides of amino acid hydrochlorides,[10] by reaction of N-protected amino acids with *p*-nitrophenyl isocyanate,[11] dialkyl pyrocarbonates in protic solvents,[12] and also through the use of well-known conventional coupling methods and reagents[12] [The terminology "conventional" or "nonconventional" is only based on the frequency of use of the coupling method reported in the literature], such as dicyclohexyl carbodiimide, mixed carboxylic carbonic anhydrides, phosphorus reagents, symmetrical anhydrides of protected amino acids, carbonyldiimidazole, and BOP reagent.

Four different coupling reagents, BOP (Castro's reagent);[13] DCC/HOBt;[14] HBTU;[14] and a slightly less conventional reagent, phosphorus oxychloride ($POCl_3$);[15] were investigated. Other coupling reagents derived from BOP (PyBOP and PyBroP) or HBTU (HATU and HCTU) were not tested because of their similar reactivity with BOP and HBTU, respectively.

Different criteria were used to compare the efficiencies of the coupling reagents and reaction conditions: nucleophilicity of the amino group conjugated to the aromatic or heteroaromatic ring, thermodynamic coupling conditions (temperature, reaction time, solvents), ease of purification of the resulting products and side products, and possibilities to be used in automated parallel synthesis.

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Various synthons were used to perform this study. Some of them were commercially available, that is, 2-amino-6-nitrobenzothiazole, 2-aminothiazole, and the 5-substituted 2-aminothiazoles, whereas the others were not, that is, the 2-substituted 5-aminotetrazoles and the 4-substituted 2-aminothiazoles. They were easily synthesized according to procedures described in the literature.

Synthesis of substituted 5-aminotetrazoles was performed in DMF to solubilize the poorly soluble 5-aminotetrazole in the presence of cesium carbonate as base (Scheme 1a).[16] After one night at 80 °C, we noticed by TLC monitoring the presence of only two compounds, which were of very different polarities. Surprisingly, each compound was identified as a N-aromatic ring substituted 5-aminotetrazole, that is, the N_1-substituted **1a** and the N_2-substituted **1b**. The major difference is in their respective ^{1}H NMR spectrum, in DMSO-d_6. The more polar compound was characterized by the presence of two main signals at 5.56 (s, CH_2) and 6.96 ppm (broad s, NH_2), whereas the other one, the less polar isomer, displayed these two characteristic signals at 5.84 and 6.14 ppm, respectively. Thanks to these data, we could unambiguously identify both isomers. Indeed, on the basis of the results previously described in the literature,[17] the less polar one was identified as the N_2-substituted isomer while the more polar one was the N_1-substituted tetrazole. Our identification was confirmed later because the more polar 5-aminotetrazole derivative (N_1-substituted) did not react with *N*-Boc-Ala in the presence of any coupling agent because of the steric hindrance of the N_1 substituent.

The spectral patterns obtained for the 4-nitrophenyl derivatives and the wide difference in polarity were similarly observed for the two other N_2-substituted 5-aminotetrazoles (**2b** and **3b**) subsequently synthesized and described herein.

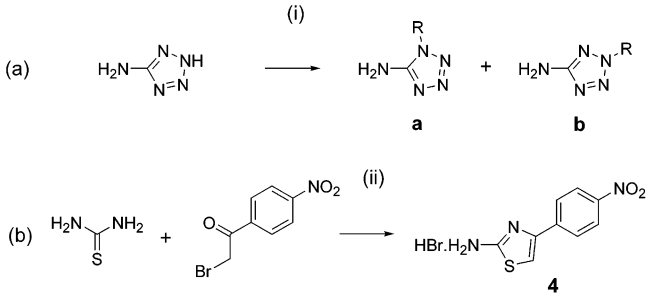
The other noncommercially available synthon used in this project was a 4-substituted 2-aminothiazole. The hydrobromide salt of the 2-aminothiazole **4**[18] was the result of a modified conventional Hantzsch synthesis,[19] achieved from α-haloketones and thiourea in refluxed ethanol. Herein, the desired compound **4** was isolated in high yield by filtration after cyclocondensation of α-bromo-4-nitroacetophenone with thiourea (Scheme 1b). This synthon, a well-known pharmacophore,[20] is generated in high yield and purity, with very low amounts of byproducts. For these reasons, 2-aminothiazole scaffold has been widely described in the literature for solution or solid-phase parallel synthesis to generate large scale libraries.[20, 21]

All these synthons were submitted to N-acylation with a protected amino acid model, *N*-Boc-Ala, under the conditions described in Table 1, and the results are summarized in Table 2.

The monitoring of the various reactions was performed by the TLC/ninhydrin detection method, which could be suitable in the case of an automated synthesis application.

Analysis of the results shows evident discrepancies among the coupling methods. As far as the carboxylic moiety (*N*-Boc-Ala) was identical for all the attempted coupling reactions, its activation by the coupling reagents ($POCl_3$, BOP, DCC/HOBt) can be assumed to be accomplished efficiently under each of the tested conditions. Taking into account this hypothesis, the next step, which is the condensation of the heterocyclic amine on the activated *N*-Boc-Ala, should be at the origin of the observed discrepancies.

Scheme 1. Synthesis of N-Substituted 5-Aminotetrazoles **1a**/**b**−**3a**/**b** and **4**-Substituted Aminothiazole 4[a]



[a] (i) RBr, $CsCO_3$, DMF, 80 °C; 30−35% in **b**, depending on the nature of R; (ii) EtOH, Δ; quantitative.

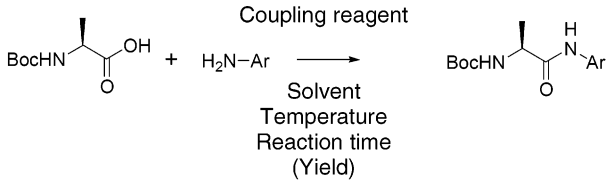
The coupling reaction between 5-amino-2-substituted tetrazoles **1b** and **2b** and *N*-Boc-Ala worked in good yields, when $POCl_3$ was used as coupling agent but totally failed when BOP or DCC/HOBt were used as activating agents. N-Acylation of 2-amino-4-(4-nitrophenyl)-thiazole **4** occurred in satisfactory yields when *N*-Boc-Ala was activated by $POCl_3$ according to a known mechanism[15] (not shown), whereas the same condensation on *N*-Boc-Ala activated with standard coupling reagents BOP or DCC/HOBt occurred only in very low yields, 19 and 5%, respectively. N-Acylation of the 2-amino-5-substituted thiazole series (compounds **9**, **10**, **11**) occurred with various yields whatever the coupling reagents used. In contrast, 2-amino-6-nitrobenzothiazole is N-acylated in high yields when amino acid activation is achieved using BOP or DCC/HOBt reagents (quantitative yields) and 76% with $POCl_3$. Yields appeared to be dependent mainly on the substituents at the 4-position of the thiazole ring, activation with $POCl_3$ being the less active coupling reagent whatever the substituents at the 5-position of the aminothiazole ring.

HBTU was assayed on two different substrates, 5-amino-2-(4-nitrobenzyl)-tetrazole **1b** and 2-amino-4-(4-nitrophenyl)-thiazole, hydrobromide salt **4**, but these conditions did not lead to the expected coupling products **5** and **8**.

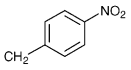
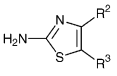
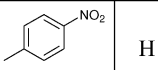
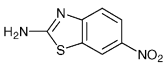
These results merit several comments. It is well-known that the rate of a nucleophilic substitution is dependent on basicity and nucleophilicity of the species involved in the reaction. In the case of a substitution reaction, nucleophilicity should be the dominant property. According to the hard−soft acid−base concept,[22] a species with a high and localized charge distribution will be hard; this pertains to both electrophiles and nucleophiles. The heteroarylamines involved in this study are borderline nucleophiles between hardness and softness and, thus, difficult to classify according to their nucleophilicity. However, it can be underlined that when $POCl_3$ is used as activating coupling reagent, the acylation is performed in pyridine as solvent, which is also a borderline nucleophile, whereas reactions carried out with BOP or DCC/HOBt as activating reagents are performed in the presence of 3 equiv of DIEA, which is a harder nucleophile than pyridine.

As far as a high solvation energy will lower the ground-state energy relative to the transition state in which the charge is more diffuse, resulting in a decreased reaction rate, in contrast, a lower-energy transition state leads to an increase in reactivity. To explain the discrepancies found in the efficiencies of the coupling reaction, it can be postulated that solvation of heterocyclic amines **1b**, **2b**, and **4** is a low-

Table 1. Yield Comparison among the Coupling Reagents, $POCl_3$/Pyridine (Method A), BOP (Method B), DCC/HOBt (Method C), and HBTU



Ar: heteroaromatic moiety

n°	H_2N-Ar			Coupling conditions: $POCl_3$/Pyridine Yield ([a])	BOP Yield ([a])	DCC/HOBt Yield ([a])	HBTU Yield ([a])
		R^1					
5				76% (RT, 18 hrs)	no reaction [b] (RT, 24 hrs)	no reaction [b] (RT, 24 hrs)	no reaction [b] (RT, 24 hrs)
6				74% (RT, 2 hrs)	no reaction [b] (RT, 24hrs)	no reaction [b] (RT, 24 hrs)	not tested
7				63% (RT, 24 hrs)	not tested	not tested	not tested
		R^2	R^3				
8			H	77% (80°C, 12 hrs)	19% (RT, 18 hrs)	5% [c] (RT, 48 hrs)	no reaction [b] (RT, 24 hrs)
9		H	Br	30% (-15°C, 4 hrs)	15% (RT, 18 hrs)	33% (RT, 2 hrs)	not tested
10		H	H	41% (RT, 3 hrs)	quantitative (RT, 48 hrs)	quantitative (RT, 24 hrs)	not tested
11		H	NO_2	58% (RT, 18 hrs)	88% (RT, 48 hrs)	84% (RT, 24 hrs)	not tested
12				76% (-15°C, 30 min.)	93% (RT, 18 hrs)	quantitative (RT, 4 hrs)	not tested

[a] Temperature, reaction time. [b] No desired compound was detected by TLC monitoring. [c] The coupling system was EDC/HOBt, in place of DCC/HOBt.

energy process in pyridine, as compared to equivalent processes in solvents such as DCM or DMF.

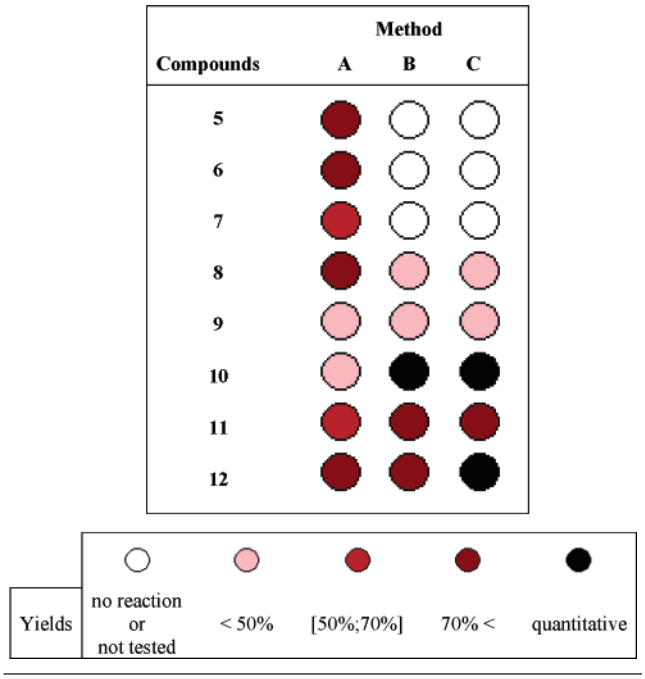
Moreover, a bulkier nucleophile will be less reactive than a smaller one because the trigonal bipyramidal geometry of the SN2 transition state is more sterically demanding than the ground state. The above results indicate that heterocyclic amines bearing the bulkier substituents compounds (**1b**, **2b**, and **4**) gave satisfactory yields when the coupling reagent was $POCl_3$, but they only slowly reacted when coupling reagents were BOP or DCC/HOBt, and no reaction occurred in the case of HBTU.

We confirmed that during the coupling reaction activated by $POCl_3$, no racemization occurred. The chiral HPLC profiles for compound **12** obtained using both coupling agents $POCl_3$ and BOP were found to be identical under both conditions and displayed only one characteristic HPLC peak. These results were compared to the similar data obtained for the corresponding racemic derivative **12(*R*,*S*)** synthesized from *N*-Boc-(*R*,*S*)-Ala and using $POCl_3$ as coupling agent. In this later case, HPLC profiles showed the presence of two peaks corresponding to the two expected isomers in identical proportions. These results are described in the Experimental Section as Supporting Information.

In summary, as depicted in Table 2, one can see that $POCl_3$/pyridine (method A) worked fine (entries **9**−**10**; clear pink dots) to well (entries **5**, **6**, **8**, **12**; dark red dots) for each studied amino substrate. On the other hand, BOP (method B) or DCC/HOBt (method C) worked well for three substrates (entries **10**−**12**; black and dark red dots) but were not efficient in some of the studied cases or were completely inefficient (entries **5**, **6**; entry **7** was not tested for methods B and C; white dots). One can imagine that, technically, this procedure involving $POCl_3$ as coupling agent will be more suitable to automation than the two other methods, the results of which are not as homogeneous as for method A ($POCl_3$/pyridine).

In conclusion, N-acylation of weakly nucleophilic heterocyclic amines by protected amino acid is not a straightforward reaction which could be achieved under any standard coupling conditions. Through the use of $POCl_3$, we were able

Table 2. Summary of the Yield Comparison among the Three Described Methodologies[a]



[a] Method A, $POCl_3$/pyridine; method B, BOP; method C, DCC/HOBt.

to perform the parallel solution-phase synthesis of new versatile adducts incorporating an amino acid model coupled to specific heterocyclic systems (substituted amino thiazole or tetrazole) bearing nitro substituents, which cannot be obtained through the use of standard activating coupling reagents. Our results indicated that when the weakly nucleophilic heterocyclic amines, to be coupled, are rather bulky (compounds **1b**, **2b**, and **4**), the use of phosphorus oxychloride ($POCl_3$) in pyridine could be an alternative method for solution-phase automated synthesis, easy to run, with reasonnable to good yields, and quite suitable for product purification (see Experimental Section in the Supporting Information) without affecting the stereochemistry. In contrast, it seems that electrodonating or -withdrawing substituents on the amino heterocyclic rings do not allow any discrimination between the coupling reagents to be used. These first very encouraging results will be the starting point of an automated synthesis of a larger library of new amino acid-heterocyclic synthons bearing nitro groups which would be then used in the design of a series of new protease inhibitors.

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Supporting Information Available. Experimental procedures and analytical data for the compounds. This material is available free of charge via the Internet at http://pubs.ac.org.

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