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Solid-phase synthesis of modified oligopeptides via Passerini multicomponent reaction

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Abstract—A Passerini multicomponent reaction of *N*-protected-α-aminoaldehydes, carboxylic acids and solid-supported isocyanides has been successfully performed on Lantern™. The initially formed *N*-protected-β-amino-α-acyloxyamides gave, by piperidine-promoted deprotection and concomitant acyl migration, β-acylamino-α-hydroxyamides which, by OH-oxidation and cleavage off the solid support, provided β-acylamino-α-oxoamides. Formation and disappearance of the isocyano group has been followed using photoacoustic IR spectroscopy, a fast, reliable and non-disruptive technique that has been successfully applied for the first time to macroscopic solid supports.

In the last decade solid-phase organic synthesis (SPOS) has attracted the attention of many research groups for a number of reasons, the main one being the ability to synthesise large collections of compounds in few synthetic steps via the split&mix technique. Beside all the reactions which have been successfully performed on the solid-phase, multicomponent reactions have become more and more popular.[1] This can be ascribed to the fact that three or more points of diversity can be generated in a single step, allowing most or all the atoms of the reactants to be included in the final adducts, reducing the time of the synthesis and giving generally clean products.

We have recently described a further exploitation of the Passerini multicomponent reaction, in which a Boc-protected α-aminoaldehyde reacts with an isocyanide and a carboxylic acid to give, after Boc deprotection and acyl migration, a β-acylamino-α-hydroxyamide;[2] compounds belonging to this class have found pharmacological applications in the field of aspartic-protease inhibitors. Furthermore, many β-acylamino-α-oxoamides, obtainable from the latter by oxidation of the secondary alcohol moiety, are known to be serine/cysteine-protease inhibitors.[3] Recently we[4] and others[5] have also shown that this novel synthetic strategy can be applied to the synthesis of known protease inhibitors: these can be obtained in less steps and better yields, in comparison with the syntheses previously reported in the literature. Hulme[6] has also reported the solution-phase synthesis of a 9,600 member library of β-acylamino-α-hydroxyamides using solid-phase scavengers.

In order to exploit this novel synthetic strategy further more, we have decided to transfer it onto the solid-phase. With this methodology, in fact, libraries of oligopeptides modified with the β-amino-α-hydroxyamide or the β-amino-α-oxoamide units can be synthesised in a very straightforward manner. Despite many papers regarding multicomponent reactions on the solid-phase, we came across only one very recent report on solid-phase Passerini reaction with supported isocyanides.[7] In this communication we report our preliminary results.

We decided to develop our strategy on the solid support Lantern™ (PS-D-AMM, 34 μmol/Lantern), in order to obtain discrete compounds in a multimilligram scale, for analytical purposes. This support, in our hands, showed a reactivity comparable to that of loose polystyrene resin, with some advantages such as easiness of handling, fixed loading and the possibility of using a coloured tagging system to univocally identify the structure of the compounds bound to the supports during split&mix synthesis. Furthermore, beside the common colorimetric tests used in solid-phase synthesis, photoacoustic IR spectroscopy (FTIR-PAS)[8] could be successfully applied for the first time. To the best of our knowledge, there is only one example in the literature of FTIR-PAS applied to solid-phase chemistry.[9]

Keywords: protease inhibitors; multicomponent Passerini reaction; solid-supported isocyanides; photoacoustic IR spectroscopy; IBX.

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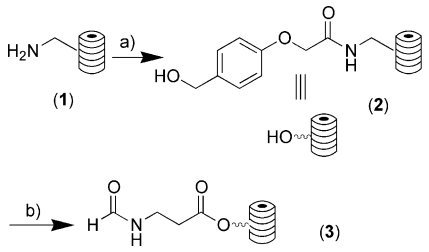
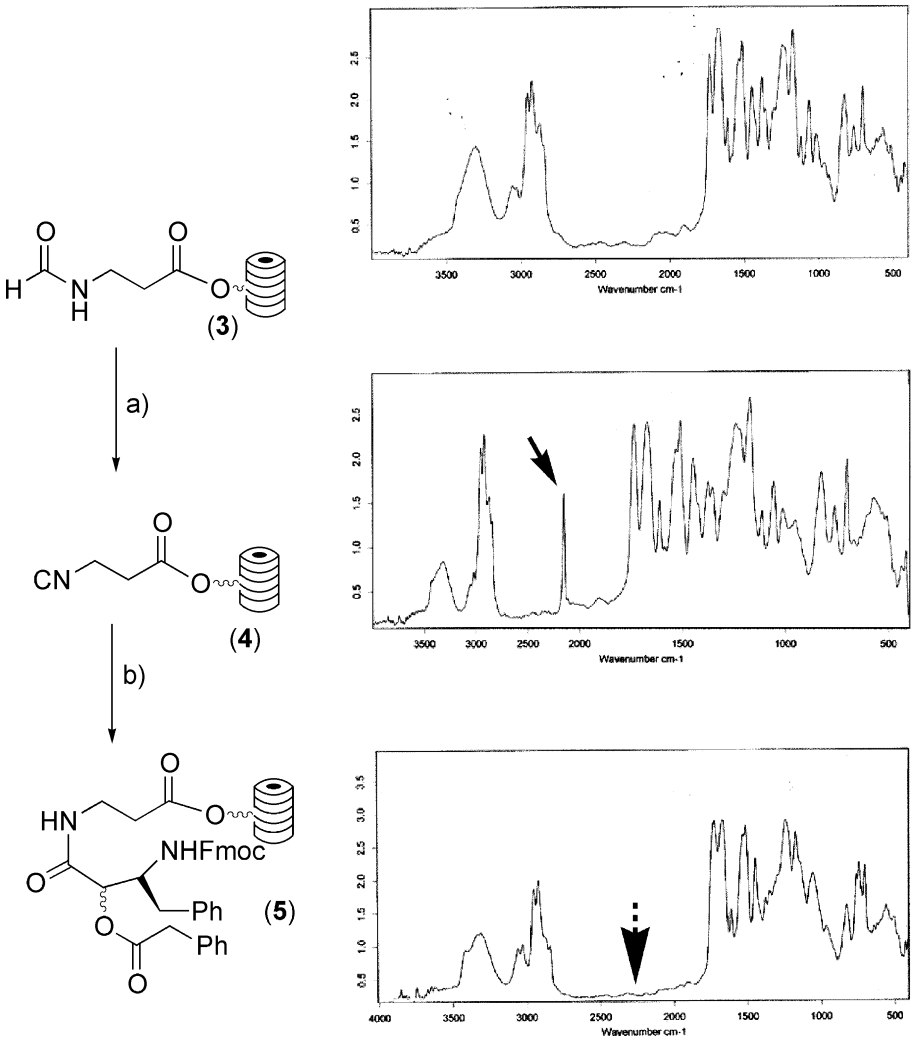


Scheme 1. *Reagents and conditions*: (a) 4-hydroxymethyl phenoxyacetic acid, DIC, HOBt, DMF; (b) 3-formilaminopropionic acid, DIC, DMAP, DMF.

However, it has never been used before for macroscopic solid supports such as Lanterns™,[10] Crowns™ or Plugs.[11] The photoacoustic signal, produced by the absorption of modulated infrared radiation by the sample in analysis and the subsequent thermal transfer to a boundary layer of a confined inert gas, is a direct measurement of the amount of absorbed radiation and can be translated into an IR spectrum by Fourier transformations. In our hands this technique was found to be fast, reliable and, last but not least, the Lantern™ could be perfectly allocated into the instrument cage, recovered after the analysis and used for the following synthetic step.

In order to obtain peptide-like molecules, we decided to use a bifunctional isocyanide, the second function being a carboxylic acid. In this manner the carboxylic group could be anchored to the solid support and the isocyano group synthesised on the solid-phase from the correspondent formylamino-group. Our synthetic strategy started from aminomethylated Lantern™ (**1**), coupled with 4-(hydroxymethyl)phenoxyacetic acid to give an HMPA-functionalised solid support (**2**) (Scheme 1).

3-Formylaminopropionic acid was attached to the solid support using DIC/DMAP and the formyl group dehydrated to isocyano with $POCl_3/Et_3N$ (Scheme 2). This reaction was followed by FTIR-PAS, by comparison of the intensity of the peak of the isocyano group at 2150 cm^{-1} with the intensity of the peaks at 1705 cm^{-1} (C=O stretching of the ester group) and 1670 cm^{-1} (C=O stretching of the amide groups). Analysing a Lantern™ (**4**) at fixed times, it was found that best conditions were 3 equiv. of $POCl_3$ and 6 equiv. of Et_3N in DCM (0.2 M



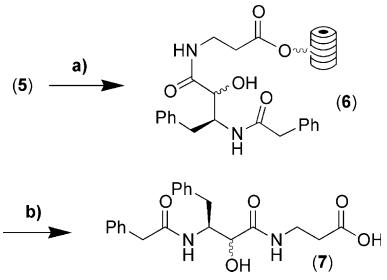
Scheme 2. *Reagents and conditions*: (a) $POCl_3$, Et_3N, DCM; (b) phenylacetic acid, Fmoc-phenylalaninal, DCM.

solution referred to $POCl_3$) at room temperature for 1.5 h. Prolonged reaction times resulted in instability of the isocyano group.

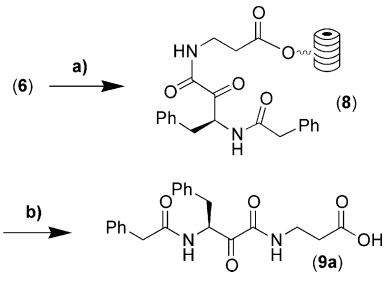
The solid supported isocyanide (**4**) was reacted with *N*-Fmoc-phenylalaninal and phenylacetic acid in DCM for 24 h. Completion of the reaction was checked by FTIR-PAS, monitoring the disappearance of the peak of the isocyano group (Scheme 2). Piperidine treatment of **5** caused Fmoc deprotection and concomitant acyl migration to form **6** which, in presence of TFA, gave rise to cleavage off the support to provide the expected β-acylamino-α-hydroxyamide (**7**) (Scheme 3). Good results in terms of HPLC purity (79%) and yield (99%) were obtained for **7**, together with the correct mass by ES-MS analysis and ^1H NMR in complete accordance with previous results obtained in solution. The diastereoisomeric ratio was found, by NMR analysis,[12] to be 6:4 as in the case of the same compound obtained in solution with a Boc or Fmoc strategy.[13]

The solid supported β-acylamino-α-hydroxyamide (**6**) was finally oxidised to give the corresponding β-acylamino-α-oxoamide (**8**). Attempts with TEMPO, TPAP, SO_3/pyridine and CrO_3 failed, only IBX[14] in DMSO giving the desired product (Scheme 4). Compound **9a** was cleaved off the resin and recovered almost quantitatively. Characterisation by NMR and ES-MS confirmed the correct structure.

With these results in hand we decided to synthesise a mini-library of such compounds (**9a–l**) varying the three components of the Passerini reaction and using a split&mix methodology. The isocyanides deriving from



Scheme 3. *Reagents and conditions*: (a) piperidine, DMF; (b) TFA, DCM.



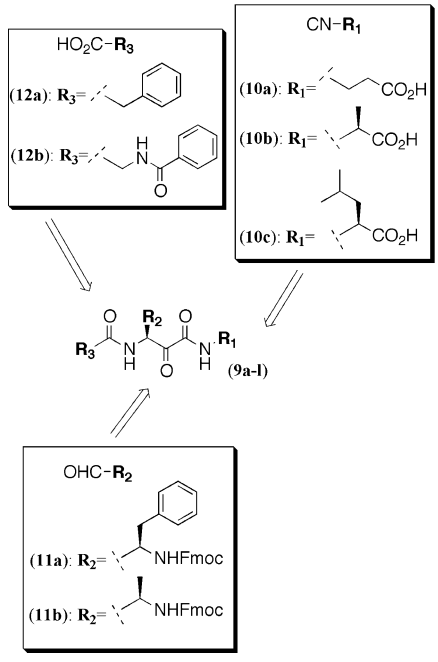
Scheme 4. *Reagents and conditions*: (a) 2-iodoxybenzoic acid, DMSO; (b) TFA, DCM.

β-alanine (**10a**), alanine (**10b**) and leucine (**10c**) were reacted with the aldehydes derived from Fmoc-phenylalanine (**11a**) and Fmoc-alanine (**11b**) and with phenylacetic (**12a**) and hippuric (**12b**) acids (Scheme 5). The isocyanides of alanine and leucine were prepared from the corresponding Fmoc-aminoacids: these were bound to the Lantern™, deprotected, formylated with 2,4,5-trichlorophenylformate and dehydrated with diphosgene/*N*-methyl morpholine.

The supported isocyanides were reacted under Passerini conditions with the four different combinations of aldehydes and acids. 12 different supports were used, one for each compound, and the history of each support was recorded thanks to a coloured tagging system. The final compounds were cleaved off the resin and purified by preparative TLC. The isolated yields ranged from good to very good, as reported in Table 1, and ES-MS analysis gave the expected results.

^1H NMR spectra of crude compounds, reported for compounds **9c** and **9d**,[15] showed in most cases substantial absence of unreacted starting materials or side reactions.

In conclusion, we have shown that a Passerini reaction/acyl migration strategy using *N*-Fmoc-protected aminoaldehydes can be successfully performed on the solid-phase and that β-acylamino-α-hydroxyamides and β-acylamino-α-oxoamides, potential protease inhibitors, can be obtained in high yields and purities. Moreover, we have applied FTIR-PAS to a macroscopic solid support for the first time. We believe that this simple technique will help to solve some of the analytical problems related to macroscopic supports and will therefore be extensively used in the near future. Further studies on the solid-phase applications of this multicomponent reaction will be published in due course.



Scheme 5. The building blocks of the 12-member test-library.

Table 1. Analytical results for the 12-member library

Prod.	Isocyanide	Aldehyde	Acid	Yield[a] (%)	ESMS$^{(-)}$
9a	**10a**	**11a**	**12a**	92	381.4
9b	**10b**	**11a**	**12a**	54	381.4
9c	**10c**	**11a**	**12a**	83	423.5
9d	**10a**	**11b**	**12a**	90	305.3
9e	**10b**	**11b**	**12a**	76	305.3
9f	**10c**	**11b**	**12a**	84	347.6
9g	**10a**	**11a**	**12b**	55	424.4
9h	**10b**	**11a**	**12b**	71	424.4
9i	**10c**	**11a**	**12b**	50	466.6
9j	**10a**	**11b**	**12b**	78	348.5
9k	**10b**	**11b**	**12b**	90	348.5
9l	**10c**	**11b**	**12b**	61	390.6

[a] Isolated yields after preparative TLC. Purity was confirmed by HPLC analysis.

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References

1. Ugi, I.; Heck, S. *Comb. Chem. HTS* **2001**, *4*, 1–34.
2. Banfi, L.; Guanti, G.; Riva, R. *Chem. Commun.* **2000**, 985–986.
3. Otto, H.-H.; Schirmeister, T. *Chem. Rev.* **1997**, *97*, 133–171.
4. Banfi, L.; Guanti, G.; Riva, R.; Basso, A.; Calcagno, E. *Tetrahedron Lett.* **2002**, *43*, 4067–4069.
5. (a) Owens, T. D.; Semple, J. E. *Org. Lett.* **2001**, *3*, 3301–3304; (b) Owens, T. D.; Araldi, G.-L.; Nutt, R. F.; Semple, J. E. *Tetrahedron Lett.* **2001**, *42*, 6271–6274.
6. Tadesse, S.; Balan, C.; Jones, W.; Viswanadhan, V.; Hulme, C.; 223rd ACS National Meeting, 2002, Orlando, FL, USA.
7. Huber, V. J.; Nagula, G.; Lum, C.; Goodman, B. A. *Abstracts of Papers of the American Chemical Society* **2001**, *221*, 140-ORGN.
8. Yan, B.; Gremlich, H. U.; Moss, S.; Coppola, G. M.; Sun, Q.; Liu, L. *J. Comb. Chem.* **1999**, *1*, 46–54.
9. Gosselin, F.; Di Renzo, M.; Ellis, T. H.; Lubell, W. D. *J. Org. Chem.* **1996**, *61*, 7980–7981.
10. Rasoul, F.; Ercole, F.; Pham, Y.; Bui, C. T.; Wu, Z. M.; James, S. N.; Trainor, R. W.; Wickham, G.; Maeji, N. J. *Biopolymers* **2000**, *55*, 207–216.
11. Atrash, B.; Bradley, M.; Kobylecki, R.; Cowell, D.; Reader, J. *Angew. Chem., Int. Ed. Engl.* **2001**, *40*, 938–941.
12. ES$^{(+)}$-MS: 385.2 ([M+H]$^+$, 100%); 407.4 ([M+Na]$^+$, 12%). ^{1}H NMR (DMSO-d_6, 90°C) *mixture of two diastereoisomers*: 2.40 (2H, t, $J=7$), 2.6–3.0 (2H, m), 3.29 (2H, quart, $J=7$), 3.38 (1.2H, s), 3.40 (0.8H, s), 3.88 (0.6H, d, $J=3$), 4.01 (0.4H, d, $J=3$), 4.2–4.4 (1H, m), 7.0–7.4 (10H, m), 7.5–7.7 (2H, m). ^{13}C NMR (DMSO-d_6) *mixture of two diastereoisomers*: 33.8, 34.2 and 34.4, 36.8, 42.1, 53.2, 71.2 and 73.3, 126.0, 126.1, 126.6, 127.9, 128.0, 128.1, 128.7, 128.9, 129.0, 129.2, 136.4 and 136.5, 136.8 and 139.1, 169.6 and 169.7, 171.6 and 171.8, 173.2.
13. Unpublished results.
14. Pearson, W. H.; Clark, R. B. *Tetrahedron Lett.* **1997**, *38*, 7669–7672.
15. *Compound* ***9c***: ^{1}H NMR ($CDCl_3$) 0.96 (6H, d, $J=5$); 1.5–1.9 (3H, m), 3.02 (1H, dd, $J=7$, 14), 3.23 (1H, dd, $J=5$, 14), 3.57 (2H, s), 4.5–4.7 (1H, m), 5.3–5.6 (2H, m), 6.07 (1H, dd, $J=8$, 10), 6.8–7.4 (10H, m).
Compound ***9d***: ^{1}H NMR (DMSO-d_6) 1.25 (3H, d, $J=7$), 2.43 (2H, t, $J=7$), 3.30 (2H, quart, $J=7$); 3.45 (2H, s), 4.95 (1H, quint, $J=7$), 7.1–7.4 (5H, m), 8.55 (1H, d, $J=7$), 8.63 (1H, t, $J=7$). ^{13}C NMR (DMSO-d_6): 197.0, 172.5, 169.9, 160.4, 136.0, 128.9, 128.1, 126.2, 49.3, 41.3, 34.7, 33.0, 15.5.