Pergamon

SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 8063–8067

TETRAHEDRON LETTERS

# Preparation of polymer-bound pyrazolone active esters for combinatorial chemistry

Jang-Woong Byun, Dong-Hoon Lee and Yoon-Sik Lee*

*School of Chemical Engineering, Seoul National University, Seoul 151-744, Republic of Korea*

Received 8 August 2003; revised 5 September 2003; accepted 8 September 2003

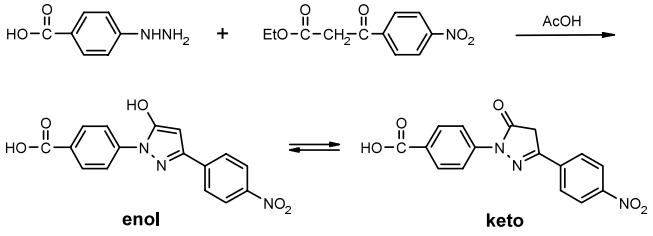
**Abstract**—The preparation of solid-phase active esters from a new pyrazolone linker resin is described. *N*-Acylation using this resin provides various amide products with a high conversion rate and good purity under mild conditions. The polymer-bound pyrazolone linkers are stable in the reaction conditions and are resistant to hydrolysis. Moreover, this resin can also be reused repeatedly without a loss of reactivity.


Polymer-bound reagents and linkers have become increasingly important tools in organic synthesis due to the growing use of combinatorial chemistry in various applications. The polymer-bound linkers have several advantages over classical solution methods. They can drive the reactions to a high conversion efficiency with an excess of reagent which can be removed simply by filtration, and can be used in multiple step synthesis and automation. In addition, a reaction using the polymer-bound linkers provides a desirable yield and purity. Therefore, a wide range of polymer-bound linkers have been developed for various organic synthesis systems.[1]

Since the preparation of peptide fragments by polymer-bound active esters have attracted a great deal of attention, several types of active esters such as nitrophenol,[2] carbodiimides,[3] *N*-hydroxysuccinimide,[4] 1-hydroxybenzotriazole,[5] pentafluorophenol,[6] and Kaiser oxime resin[7] have been reported. During our continuing efforts for developing versatile polymer-bound active ester linkers, we became interested in pyrazolone active esters for *N*-acylations in combinatorial chemistry.[8]

Compared to other reagents, polymer-bound pyrazolone active esters are stable in polar solvents such as alcohols and DMF, and are resistant to hydrolysis. Furthermore, they can react specifically with amines under mild conditions when hydroxyl groups are present in the same solution. The progress of the reaction can be easily monitored by the change in the resin color. These characteristics of pyrazolone active esters can allow a wide structural diversity in a combinatorial library synthesis and attractive methods for modifying biomolecules. This paper reports the preparation of polymer-bound pyrazolone active esters and their applications as *N*-acylating agents in a combinatorial library synthesis.

As illustrated in Scheme 1, the desired pyrazolone linker, 1-(4-carboxyphenyl)-3-(4-nitrophenyl)-2-pyrazolin-5-one (CNPO), which possesses an anchoring group to introduce its functionality onto the polymer support, was synthesized from 4-hydrazinobenzoic acid and 4-nitrobenzoylacetate by a modified Knorr's method.[9] Due to the poor solubility of 4-hydrazinobenzoic acid in most organic solvents, acetic acid was the only choice for the reaction solvent, which also acted as an acid catalyst. As the reaction progressed under refluxing conditions, the reaction mixture was completely dissolved in a short time due to the formation of an intermediate adduct. Eventually, the CNPO began to precipitate as a yellow solid.[10] In solution, CNPO




**Scheme 1.** Preparation of the pyrazolone active ester linker (CNPO).

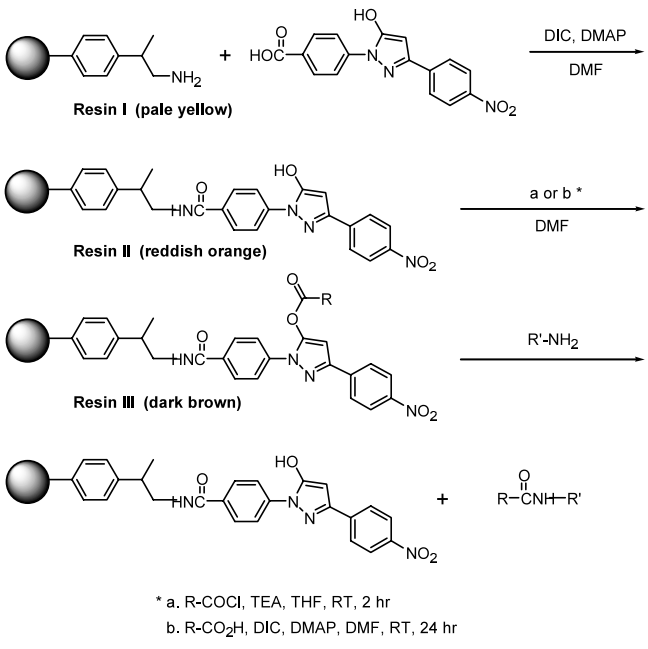
* Corresponding author. Tel.: +82-2-880-7073; fax: +82-2-876-9625; e-mail: yslee@snu.ac.kr

0040-4039/$ - see front matter © 2003 Published by Elsevier Ltd.
doi:10.1016/j.tetlet.2003.09.044

might be tautomerized between the enol and keto forms. However, NMR spectroscopy confirmed that the enol form was predominant in the polar solvent.

An amino polystyrene (PS) resin was chosen as the support for the active esters, because it could form a chemically stable amide bond with the pyrazolone linker. The amino PS resin was prepared from the PS-PO resin (BTCore OH resin from BeadTech Inc.) as reported previously.[11] After the CNPO was coupled with the amino resin (Resin I, 1.47 mmol $NH_2$/g) using DIC (3.0 equiv.) and DMAP (0.3 equiv.) in DMF, the pyrazolone containing polymer support (Resin II) was obtained as a reddish orange resin[12] (Scheme 2). From FT-IR analysis of Resin II, the amide carbonyl band at 1650 $cm^{-1}$ appeared as a result of coupling (Fig. 1a). A picric acid titration of Resin II revealed that a trace amount of amino groups (8.7 μmol $NH_2$/g) remained unreacted, which shows that the coupling yield of CNPO linker with the amino resin was approximately 99%. Finally, the degree of substitution of the CNPO linker on Resin II was 1.01 mmol/g.

The polymeric active ester (Resin III) was prepared from a coupling reaction of Resin II with several kinds of the acyl derivatives (Fig. 2). The acyl halides such as benzoyl chloride (**1**) and phenylacetyl chloride (**2**) were reacted in 2% TEA/THF (v/v) for 2 h.[13] The carboxylic acid derivatives such as 4-nitrobenzoic acid (**3**), and 4-dimethylaminobenzoic acid (**4**), and amino acids such as Boc-Gly (**5**) and Boc-Phe (**6**) were reacted with DIC (3.0 equiv.) and DMAP (0.3 equiv.) in DMF for 24 h.[14] As a result of the acyl coupling reaction, the color of the resin changed from reddish orange to a dark brown, and the carbonyl band at 1790 $cm^{-1}$ appeared (Fig. 1b).




**Scheme 2.** Preparation of the polymer-bound pyrazolone active esters and the combinatorial synthesis of amide derivatives using them.

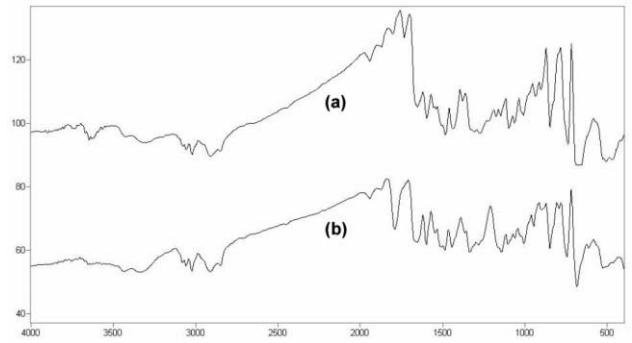



**Figure 1.** FT-IR spectra of (a) CNPO linked amino PS resin (Resin II), and (b) *O*-acetylated pyrazolone resin (Resin III).

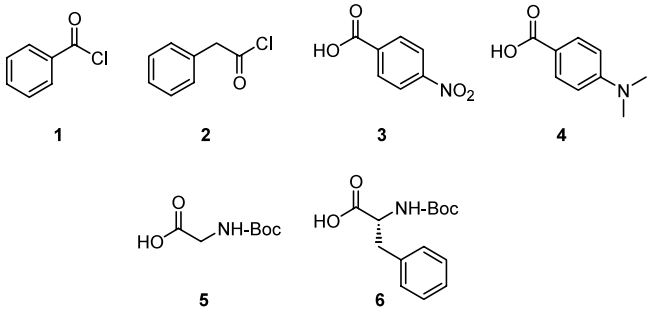



**Figure 2.** Acyl derivatives for *N*-acylation.

The degree of acylation on Resin III was determined by the benzylamine treatment[15] (Table 1). Even if the acylation onto the CNPO linker was carried out with an excess of the acyl derivatives for a long reaction time, approximately 50% of the pyrazolone groups were available for coupling, because steric hindrance of the acyl products on the PS backbone prevented further diffusion of the acylating reagents. The reactivity of the acyl derivatives also had an effect on the coupling reactions because the conversion was different according to their reactivity. Overall, the acyl chlorides (**1**, **2**) gave better results than the carboxylic acids due to their higher reactivity. In the case of compound **4**, the electron donating effect of the *p*-dimethylamino group might reduce the reactivity, and showed poor coupling.

When the amino acids (**5**, **6**) were coupled, the bulkiness of the side chain appeared to control the reactivity. Therefore, the less sterically hindered Boc-Gly was coupled more to the CNPO linker than Boc-Phe.

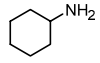
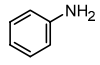
Several amide derivatives were synthesized by a reaction of Resin III with various organic amines which have a similar molecular weight.[16] The results of the combinatorial *N*-acylation using the polymer-bound pyrazolone active esters are summarized in Table 2. The progress of the reaction was easily monitored by the change in the resin color as well as by FT-IR spectroscopy. As the reaction progressed, the color of

**Table 1.** Preparation of the acylated CNPO resin (Resin III)

| Acyl derivatives | Theoretical loading of acyl derivatives on Resin III (mmol/g) | Loading of acyl derivatives on Resin III (mmol/g)[c] | Conversion of acylation (%) |
|---|---|---|---|
| **1**[a] | 1.01 | 0.76 | 75.3 |
| **2**[a] | 1.00 | 0.60 | 59.6 |
| **3**[b] | 0.88 | 0.43 | 49.2 |
| **4**[b] | 0.88 | 0.19 | 21.1 |
| **5**[b] | 0.87 | 0.46 | 52.3 |
| **6**[b] | 0.81 | 0.28 | 34.9 |

[a] The coupling reactions of the acyl chlorides (3.0 equiv.) were carried out in 2% TEA/THF (v/v) at room temperature for 2 h.
[b] The coupling reactions of the carboxylic acid derivatives (3.0 equiv.) were carried out with a mixture of DIC (3.0 equiv.) and DMAP (0.3 equiv.) in DMF at room temperature for 24 h.
[c] The loading was calculated from the amount of *N*-acyl benzylamine after a treatment with excess benzylamine (10 equiv.) at room temperature for 1 h.

**Table 2.** Combinatorial synthesis of the amide derivatives using the polymer-bound pyrazolone active esters[a]

| Entry | Amine derivatives[b] | Acyl derivatives[c] | | | | | |
|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** | **5** | **6** |
| 1 |  | 99.7 % | 99.6 % | 89.3 % | 61.1 % | 83.8 % | 78.2 % |
| 2 |  | 93.3 % | 85.0 % | 86.1 % | 55.9 % | 79.1 % | 74.8 % |
| 3 |  | 3.9 % | 49.3 % | 4.1 % | 56.8 % | 9.6 % | 8.6 % |
| 4 |  | NR | NR | NR | NR | NR | NR |

[a] All the reactions were carried out in THF at room temperature for 1 hr.
[b] 3.0 eq. of amine derivatives were reacted.
[C] The isolation yields are based on the loading of the acyl derivatives on the polymer-bound CNPO linker (0.3 g).

the resin changed from dark brown to a reddish orange, and the carbonyl band at 1790 $cm^{-1}$ completely disappeared. The remaining amine was removed by passing it through a silica gel short column. After evaporating the solvent, most of the amide products were obtained as pure solids which were confirmed by NMR. The product yields were based on the loading of acyl derivatives on the polymer-bound pyrazolone linker (Resin III).

This study found that the rate of *N*-acylation was largely dependent on the reactivity of the amine derivatives. Amines with high nucleophilicity such as piperidine (entry 1) and cyclohexylamine (entry 2) showed good results of *N*-acylation.

However, diisopropylamine (entry 4) and aniline (entry 5) barely reacted with the pyrazolone active esters due to their poor nucleophilicity.

In case of Boc-Phe derivatives, we examined the degree of racemization during *N*-acylation after treatment with (*R*)-α-methylbenzylamine. From the HPLC analysis data, we found that the degree of racemization was approximately 14.8% during the acyl coupling with DIC and DMAP for 24 h.[17] However, when we carried out the acyl coupling with BOP (3.0 equiv.), HOBt (3.0 equiv.) and DIEA (6.0 equiv.) at room temperature for 12 h, the racemizaiton was significantly reduced (<1.5%).

Repeated tests were performed to examine the stability of the polymer-bound CNPO linker. Figure 3 shows the results of the same resin, which was used repeatedly in the *N*-acylation five times with the previous benzoyl chloride and piperidine. These results showed that the CNPO linker resin (Resin III) could be reused continuously in *N*-acylation without changing its reactivity.

In conclusion, a polymer-bound pyrazolone active ester resin can be used successfully with a high reactivity and good reusability, which makes it suitable candidate as a new polymeric acylating agent in solid-phase combinatorial chemistry.

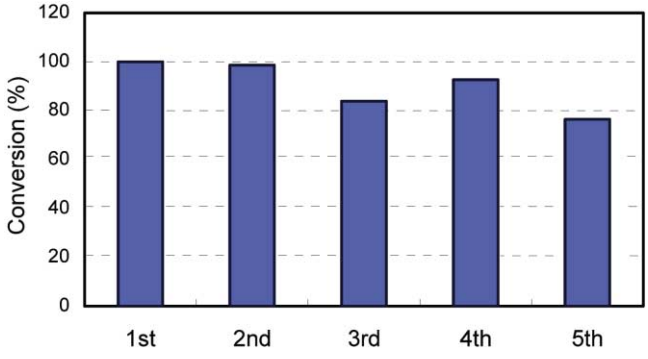



**Figure 3.** Reusability test of the CNPO linker resin.

## Acknowledgements

We wish to acknowledge the Brain Korea 21 Program supported by the Ministry of Education and the Nano Bioelectronics and Systems Research Center of Seoul National University, which is supported by the Korean Science and Engineering Foundation (KOSEF).

## References

1. For a recent review, see: James, I. W. *Tetrahedron* **1999**, *55*, 4855–4946.
2. (a) Fridkin, M.; Hazum, E.; Kalir, R.; Rotman, M.; Koch, Y. *J. Solid-Phase Biochem.* **1977**, *2*, 175–182; (b) Carpino, L. A.; Cohen, B. J.; Lin, Y. Z.; Stephens, K. E., Jr.; Triolo, S. A. *J. Org. Chem.* **1990**, *55*, 251–259; (c) Cohen, B. J.; Karoly-Hafeli, H.; Patchornik, A. *J. Org. Chem.* **1984**, *49*, 922–924; (d) Reichwein, J. F.; Liskamp, M. J. *Tetrahedron Lett.* **1998**, *39*, 1243–1246; (e) Chang, Y.-T.; Schultz, P. S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2479–2482; (f) Kim, K.; Le, K. *Synlett* **1999**, *12*, 1957–1959.
3. (a) Weinshenker, A.; Shen, C. M. *Tetrahedron Lett.* **1972**, *14*, 3281–3284; (b) Desai, M. C.; Stephens Straminello, L. M. *Tetrahedron Lett.* **1993**, *34*, 7685–7688.
4. Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1963**, *85*, 3039.
5. (a) Kalir, R.; Warshawsky, A.; Fridkin, M.; Patchornic, A. *Eur. J. Biochem.* **1975**, *59*, 55–61; (b) Mokotoff, M.; Patchornic, A. *Int. J. Pept. Protein Res.* **1983**, *21*, 145–154; (c) Mokotoff, M.; Zhao, M.; Roth, S. M.; Slavosky, J. N.; Shelley, J. A. *J. Med. Chem.* **1990**, *33*, 354–360; (d) Pop, I. E.; Deprez, B. P.; Tartar, A. L. *J. Org. Chem.* **1997**, *62*, 2594–2603; (e) Dendrinos, K. G.; Kalivretenos, A. G. *Tetrahedron Lett.* **1998**, *39*, 1321–1324; (f) Dendrinos, K.; Jeong, J.; Kalivrenos, A. G. *Chem. Commun.* **1998**, 499–500; (g) Schiemann, K.; Showalter, H. D. H. *J. Org. Chem.* **1999**, *64*, 4972–4975.
6. Kovacs, J.; Kisfaludy, L.; Ceprini, M. Q. *J. Am. Chem. Soc.* **1967**, *89*, 183.
7. (a) Smith, R. A.; Bobko, M. A.; Lee, W. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2369–2374; (b) Golebiowski, A.; Klopfenstein, S. *Tetrahedron Lett.* **1998**, *39*, 3397–3400; (c) Scialdone, M. A.; Shuey, S. W.; Soper, P.; Hamuro, Y.; Burns, D. M. *J. Org. Chem.* **1998**, *63*, 4802–4807.
8. (a) Lee, K. W.; Lee, Y. S. *Bull. Korean Chem. Soc.* **1989**, *10*, 331–335; (b) Kim, J. B.; Lee, Y. S. *Bull. Korean Chem. Soc.* **1991**, *12*, 376–379.
9. Knorr, A. *Ber. Deutsch. Chem. Ges.* **1950**, *16*, 2597.
10. **A general procedure for the CNPO synthesis**. 4-Hydrazinobenzoic acid (6.09 g, 40.0 mmol) and 4-nitrobenzoylacetate (10.25 g, 48.0 mmol) were mixed in acetic acid (200 ml), and refluxed for 24 h. After the reaction was completed, the reaction mixture was cooled to room temperature. The CNPO was obtained as a brown solid, which was filtered and washed thoroughly with DCM to the remove excess acetic acid. Yield, 10.78 g (83%); mp 274°C; NMR (DMSO-$d_6$) $\delta$ 4.4 (s, -$CH_2$-), 6.6 (s, -OH), 8.2–8.8 (m, aromatic).
11. Ryoo, S. J.; Kim, J.; Kim, J. S.; Lee, Y. S. *J. Comb. Chem.* **2002**, *4*, 187–190.
12. **A general procedure for the preparation of CNPO linked amino PS resin (Resin II)**. Resin I (8.0 g, 1.47 mmol -$NH_2$/g) was swelled with DMF in a three-necked round-bottomed flask. The mixture of CNPO (11.4 g, 35.3 mmol), DIC (5.5 ml, 35.3 mmol) and DMAP (422.5 mg, 3.5 mmol) in DMF was then added and stirred for 24 h. The product resin was filtered and washed three times each with DMF, MeOH and DCM in turn, and then dried in a vacuum.
13. **A general procedure for the coupling of the acyl chlorides to the polymer-bound CNPO linker (Resin III)**. Resin II (3.0 g, 1.01 mmol CNPO/g) was treated with benzoyl chloride (1.2 ml, 9.1 mmol) in 2% TEA/THF (v/v) and shaken for 2 h. The resin was washed three times each with DCM and MeOH, and dried under vacuum.
14. **A general procedure for the coupling of the caboxylic acid derivatives to the polymer-bound CNPO linker (Resin III)**. Resin II (3.0 g, 1.01 mmol CNPO/g) was treated with a mixture of 4-nitrobenzoic acid (1.5 g, 9.1 mmol), DIC (1.6 ml, 10.0 mmol) and DMAP (36.7 mg, 0.9 mmol) in DMF and shaken for 24 h. The resin was washed three times each with DCM and MeOH, and then dried in vacuum.
15. To determine the degree of substitution, the acylated CNPO-Resin III (0.1 g) was swelled in THF, and treated with excess benzylamine (1.0 ml, 9.2 mmol, 10 equiv.). After reacting for 1 h at room temperature, the resin was filtered and washed with DCM. The filtrate and the washing solution were combined and passed through a silica gel short column to remove the remaining benzylamine. After evaporating the solvent, the degree of acylation on th polymer-bound CNPO linker was calculated from the amount of the isolated *N*-acyl benzylamine. The quantitative conversion was confirmed by FT-IR spectroscopy, which showed that the carbonyl band of active esters (1790 $cm^{-1}$) had completely disappeared.
16. **A general procedure for the combinatorial synthesis of the amide derivatives**. Benzoyl-CNPO-Resin III (0.3 g, 0.76 mmol/g) was swelled with DCM in a filtered vial. Piperidine (77.4 μl, 0.78 mmol) in THF (5 ml) was transferred into the reaction vessel and the reaction mixture was shaken for 1 h at room temperature. The resin was filtered and washed with DCM. The filtrate and the washing solution were combined and passed through a silica gel short column to remove the excess amine derivatives. After evaporating the solution, the amide product

was obtained as a white solids (43.2 mg) and the yield was 99.7% based on the amount of the loaded benzoyl active ester groups.

17. To determine the degree of racemization, Boc-L-Phe-CNPO-Resin III (0.1 g, 0.28 mmol/g) was swelled in THF, and treated with excess of (*R*)-α-methylbenzylamine (40 μl, 0.28 mmol, 10 equiv.). After reacting for 1 h at room temperature, the filtrate and the washing solution were combined and passed through a silica gel short column to remove the remaining (*R*)-α-methylbenzylamine. After evaporating the solvent, the product was dissolved in MeOH and analyzed by HPLC using a Supelcosil LC-18 column (4×150 mm). The elution condition was as follows: 0 to 5 min, $CH_3CN/H_2O$ (50/50); 5 to 25 min, up to $CH_3CN/H_2O$ (90/10, gradient); 25 to 40 min, $CH_3CN/H_2O$ (90/10); 1.0 ml/min, 210 nm UV. The retention time of (*S*,*R*)-Boc-Phe-α-methylbenzylamide and (*R*,*R*)-Boc-Phe-α-methylbenzylamide were 13.7 and 13.2 min, respectively.