Pergamon

Tetrahedron Letters, Vol. 35, No. 32, pp. 5825-5828, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01204-0

Post-Modification of Peptoid Side Chains: [3+2] Cycloaddition of Nitrile Oxides with Alkenes and Alkynes on the Solid-Phase.

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Key words: chemical diversity, solid-phase synthesis, [3+2] cycloaddition, nitrile oxides, isoxazole, isoxazoline.

Abstract: A series of isoxazoles and isoxazolines were synthesized on solid-phase through [3+2] cycloaddition reactions of alkynes and alkenes with highly reactive nitrile oxides.

Chemical diversities on solid-phase have attracted tremendous attention because of their potential application in rapid drug discovery.[1] Among the reported approaches, robotically generated equimolar mixtures of oligo(*N*-substituted glycines) (peptoids) have proven to be advantageous for their wide range of diversity, ease of synthesis and proteolytic stability.[1a, 2] Novel drug leads have been identified through this method.[3] Isoxazole and isoxazoline moieties represent a class of unique pharmacophores which are observed in many therapeutic agents.[4] Therefore, it is meaningful to introduce these functionalities into peptoid libraries to enhance the possibility of discovering new drug leads. We have reported the multi-step regio-selective synthesis of a series of aminomethyl-isoxazoles[5] which were incorporated into peptoids through submonomer solid-phase synthesis. Herein reported is a one-step formation of isoxazoles and isoxazolines through [3+2] cycloaddition of nitrile oxides with alkynes and alkenes on the solid-phase. This post-modification strategy has the potential to be extended to other types of reaction for altering peptoid side-chains and may allow greater chemical diversity. In addition, [3+2] cycloaddition reactions of nitrile oxides in solution are known to suffer from many side reactions due to their high reactivity.[6] Therefore, it is synthetically significant to carry out these reactions on the solid-phase which makes isolation of the products easier.

Rink amide resin [7] (acid labile, 0.55 mmol/g) was used as the solid support in our experiments. Resin-bound peptoids **1** through **5** were prepared through the submonomer method according to the reported procedure[1a] with necessary modifications [8]. As shown in Table 1, the [3+2] cycloaddition reactions were carried out under two different conditions, in toluene at 100 °C [9] or in CH_2Cl_2/H_2O at room temperature, depending upon the precursors of the nitrile oxides . Benzaldehyde oxime and various nitroalkyl compounds were selected as nitrile oxide precursors. Nitrile oxides were generated *in situ* by reacting the nitroalkyl compounds with phenyl isocyanate and triethylamine or by oxidizing the oximes with sodium hypochlorite in the presence of triethylamine. These nitrile oxides are representative for their instability (due to the lack of steric hindrance), and are known to give rise to various byproducts under [3+2] cycloaddition reaction conditions.[6] In our case, all side-reactions of these nitrile oxides took place in the liquid phase and the byproducts were removed by washing the resin with appropriate solvents. The products were cleaved from the resin by treatment with 20% TFA in CH_2Cl_2 at room temperature. In all cases (except eq. 4 and eq. 9), HPLC analyses indicated >80% purity of desired isoxazoles and isoxazolines and no major byproducts were observed. Therefore, it is reasonable to assume that the overall yields of the [3+2] adducts are in the same range as their purity. The experimental procedure for the preparation of **1a** is given as an example.[12] The analytical data of **1a** are also included (Figure 1).

Resin bound peptoid **1**, with an alkyne side chain, was first used as a model to investigate the applicability of the [3+2] cycloaddition reactions of nitrile oxides on the solid-phase. As shown in Table 1, the [3+2]

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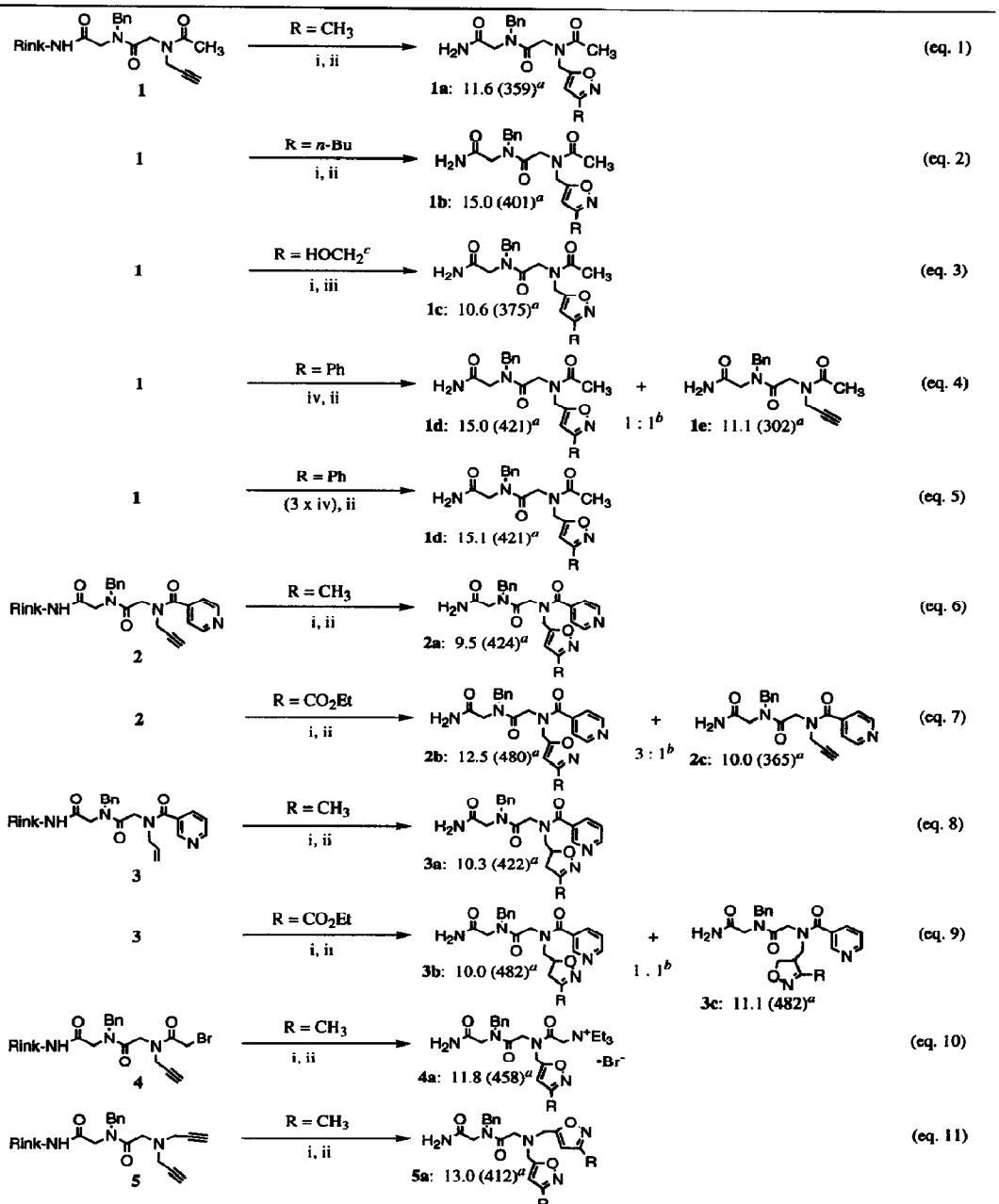
Table 1: [3+2] Cycloaddition Reactions of Nitrile Oxides on Solid-Phase.



(i) RCH_2NO_2, Ph-N=C=O, Et_3N, toluene 100 °C. (ii) 20% CF_3CO_2H in CH_2Cl_2, rt. (iii) 95% CF_3CO_2H in H_2O, rt. (iv) RCH=NOH, NaOCl, Et_3N, CH_2Cl_2/H_2O, rt. [a] HPLC retention time in minutes [10]; the number in brackets is (MH^+) [11]. [b] The ratio was determined by HPLC equipped with a UV detector at 214 nm. [c] from the corresponding THP protected ether.

cycloaddition reaction of **1** worked well with various nitrile oxides under both reaction conditions (eq. 1 through eq. 5). Single regioisomers of isoxazoles and isoxazolines (**1a** though **1d**) were obtained respectively. In eq. 3, 95% TFA was used in the cleavage step to ensure the complete removal of the tetrahydropyran (THP) protecting group. The incomplete cycloaddition in eq. 4 was due to the rapid decomposition or dimerization of the nitrile oxide. Complete conversion of **1** to **1d** was achieved by repeating the [3+2] cycloaddition step (3 x) before the TFA cleavage (eq. 5). Peptoid **2** has an isonicotinic acid capping group. It also underwent [3+2] cycloadditions with nitrile oxides in a regioselective manner to give **2a** and **2b**, respectively (eq. 6 and eq. 7). The incomplete cycloaddition in eq. 7 was believed to be due to the rapid decomposition or dimerization of the nitrile oxide from ethyl nitroacetate. Peptoid **3**, with an alkene side chain, gave a single isomer of isoxazoline with the nitrile oxide from nitroethane (eq. 8). However, it furnished a mixture of two regioisomers (1 : 1) with the nitrile oxide from ethyl nitroacetate (eq. 9).[13] It has been documented that electron-withdrawing groups (in this case $-CO_2Et$) decrease the regioselectivity and stability of nitrile oxides (eq. 9 and eq. 7, respectively).[6] The *N*-terminal of peptoid **4** was capped with a bromoacetyl group (eq. 10). In addition to the formation of isoxazole, triethylamine displaced the bromide and gave a quaternary ammonium salt under these reaction conditions. Peptoid **5** has an *N,N*-dipropargyl terminus; it gave the bis(isoxazole) product cleanly with nitrile oxide from nitroethane (eq. 11).

In conclusion, the post-modification of peptoid side chains on the solid-phase has been developed using [3+2] cycloaddition reactions of nitrile oxides to introduce isoxazole and isoxazoline moieties. This approach and other reaction types that alter peptoid side chains have the potential to create greater chemical diversity than might otherwise be possible.

Acknowledgments: The authors wish to thank Dr. Surinder Kaur and Mr. Dazhi Tang at Chiron Corporation for their assistance with mass spectrometry analysis. We also wish to thank Dr. Edward Brown, Dr. Dane Goff, Dr. Lutz Richter, Dr. Reyna Simon, Dr. Kerry Spear and Dr. Ronald Zuckermann at Chiron Corporation for their helpful suggestions and discussions.

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8. The *N*-termini of these resin-bound peptoids were capped with various acyl groups. When $R^3 = CH_3$, the acylation reaction was carried out with acetic anhydride in the presence of N,N-diisopropylcarbodiimide (DIC). However, DIC activation was ineffective with nicotinic acid and isonicotinic acid (R^3 = 3-pyridinyl and 4-pyridinyl, respectively). In these cases, complete cappings were achieved using benzotriazol-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBop)/*N*-hydroxybenzotriazole (HOBt)/DIEA as activating reagents.
9. [3+2] Cycloaddition reactions were incomplete when they were carried out with ultrasound instead of heating at 100 °C.
10. HPLC analyses were carried out on a Waters HPLC system (600E system controller, 991 photodiode array detector) with a C-18 reverse-phase HPLC column (Vydac, 25 cm x 0.46 cm) and a linear gradient elution at flow rate of 1.60 ml/min. (solvent A: H_2O/0.1% TFA; solvent B: CH_3CN/0.1% TFA; 5-85% B in 23 minutes) followed by 100% B for 7 minutes. Individual peaks off the HPLC column were collected, dried on a centrifugal concentrator and submitted for mass spectrometry.
11. Ion spray mass spectrometry was performed on a PE Sciex API *III* Biomolecular Mass Analyzer.
12. A typical experimental procedure for the [3+2] cycloaddition reaction of nitrile oxides on the solid-phase is described below. To a round-bottomed flask containing a suspension of dried resin-bound peptoid **1** (100 mg, 0.055 mmol) in toluene (5 ml) under argon was added nitroethane (0.040 ml, 0.55 mmol), phenylisocyanate (0.120 ml, 1.10 mmol) and triethylamine (0.153 ml, 1.10 mmol). The mixture was stirred at 100 °C for 5 hours. The resin was collected by filtration, washed with DMF (2 ml x 3), CH_2Cl_2 (2 ml x 3), and air dried for 15 minutes. The washes with DMF are essential to remove the *N,N'*-diphenylurea formed as a byproduct of the reaction. The dried resin was treated with 20% TFA in CH_2Cl_2 at room temperature for 30 minutes. The resin was removed by filtration. The filtrate was collected in a conical tube and concentrated under an argon stream. The residue was lyophilized from acetic acid (5 ml) to give **1a** as a white powder (very hygroscopic, 14.3 mg, 72% based on the molar equivalent of the resin). **1a**: ^1H NMR ($CDCl_3$, 300 MHz) δ 2.23(s, 3H), 2.28(s, 3H), 4.06(s, 2H), 4.13(s, 2H), 4.63(s, 2H), 4.68(s, 2H), 6.08(s, 1H), 6.87(bs, 2H), 7.22-7.37(m, 5H). IR(neat): 3325(b), 3202, 2934, 1775, 1655, 1474, 1420, 1217, 1175, 1002, 956, 702 cm^{-1}.

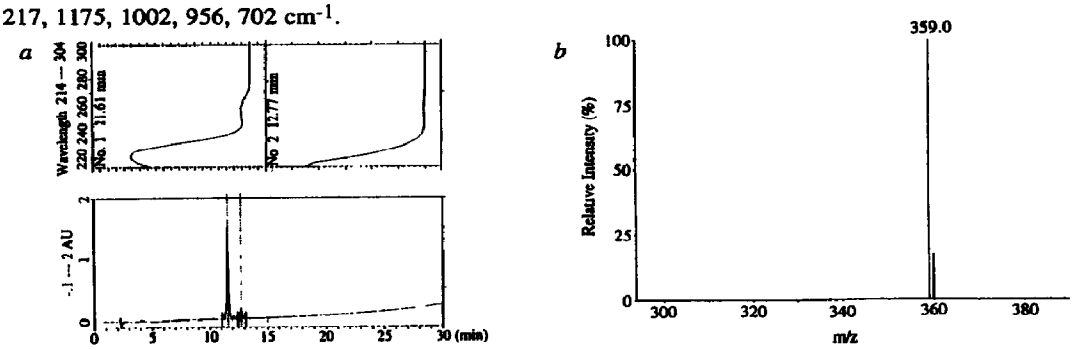

Figure 1: *a*, HPLC trace of the crude **1a**. *b*, mass spectrum of purified **1a**.

13. The regiochemistry assignments of these two isomers are arbitrary.

(*Received in USA* 31 *May* 1994; *revised* 14 *June* 1994; *accepted* 16 *June* 1994)