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Surprising behavior of NXO-peptides toward the lithium hydroxide solvolysis

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ABSTRACT

An unexpected rearrangement of NXO peptides was observed during solvolysis of the methyl ester using lithium hydroxide as the base. It was found that the NXO-compounds rearranged into semioxamazide derivatives in which the ester is derived from the alcohol used as the reaction solvent.

Peptidomimetics have emerged as an important synthetic tool for drug discovery[1] to circumvent some of the problems associated with natural peptides. Peptide mimetics are designed to mimic the biological activity of peptides while offering the advantages of increased bioavailability, biostability, bioefficiency, and bioselectivity against the natural biological target of the parent peptide.[2] Amino acids, being the smallest repeating elements of peptides, are the obvious candidates for structural and chemical exploitation toward peptidomimetics. Extensive research efforts have been focused on developing modified amino acid building blocks which can improve stability profiles and the pharmacokinetic properties of the parent peptide.[3] However, these have not been able to keep pace with the emergence of new potential binding targets and the search for new methods continues.

In an endeavor to create new building blocks for peptide modification, we have developed NXO building blocks (Fig. 1) via modification of amino acids by introducing an oxalic acid functionality at the N-terminus and a hydrazine functionality at the C-terminus.[4] NXO compounds are easy to prepare and can mimic the acceptor–donor pattern of a natural tripeptide (Fig. 2). Furthermore, NXO modification can impart conformational constraints on the peptide sequence to give new pseudo peptides selectively adopting predictable secondary structures.[4] NXO-amino acids have proved to be valuable as building blocks which can be integrated efficiently into peptide fragments using both conventional liquid phase peptide synthesis and solid supported synthesis protocols.[4]

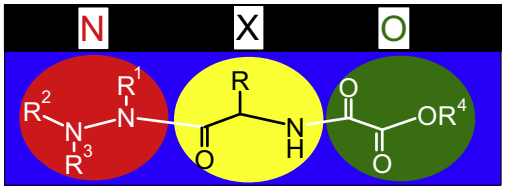

Figure 1. General formula for NXO-compounds. Where X = amino acid, R^1–R^4 = H, alkyl, aryl, protecting groups.

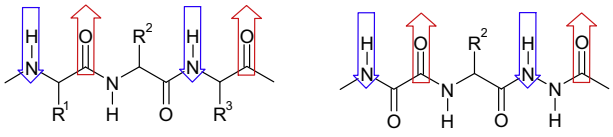

Figure 2. The donor–acceptor pattern of a tripeptide (left) versus an NXO-peptide.

During studies performed to assess the efficiency and compatibility of orthogonally protected NXO building blocks for integration into peptide fragments using different coupling agents and solvents, methyl ester solvolysis of compound **1** repeatedly produced **2** as a side product (Scheme 1).

A simple and efficient method for hydrolysis of methyl ester is typically saponification with aqueous alkali, in the presence of organic solvents such as methanol, ethanol, dimethylformamide, 1,4-dioxane etc.[5] The structure of rearranged product **2** was

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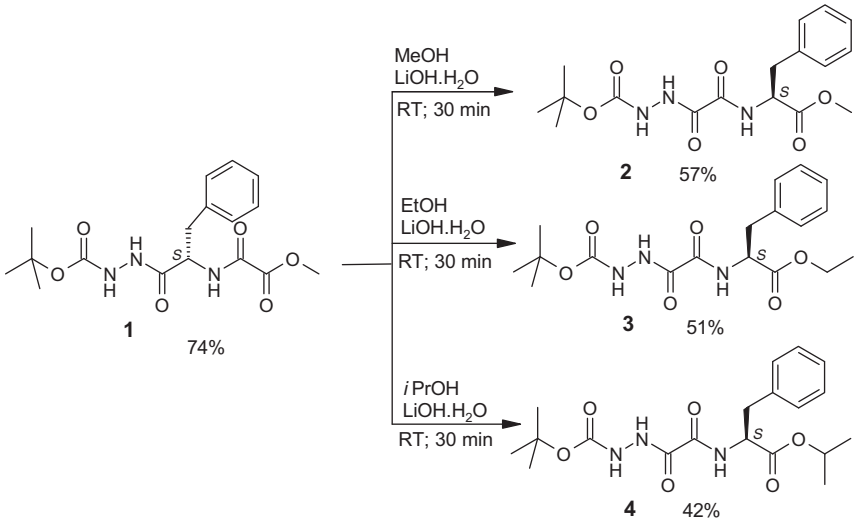
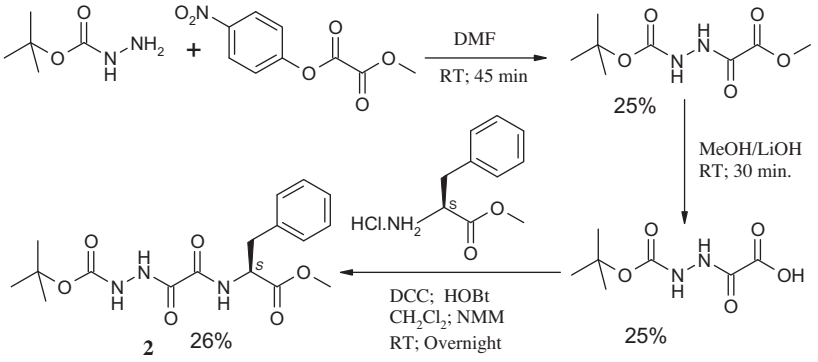


Scheme 1. Solvolytic rearrangement of NXO-compounds using different solvents.



Scheme 2. Alternative route for the synthesis of the rearranged product **3**.[6]

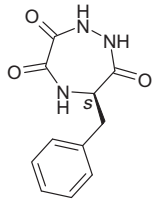
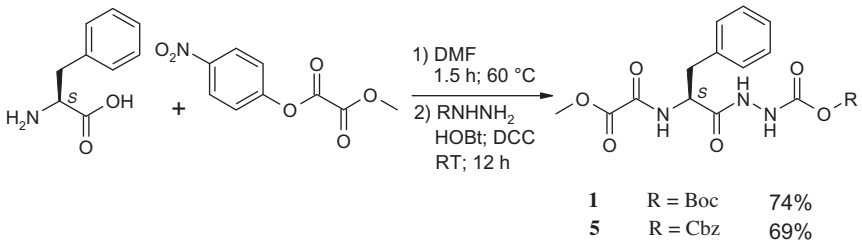


Figure 3. Proposed cyclized structure of the NXO-compound.[7]

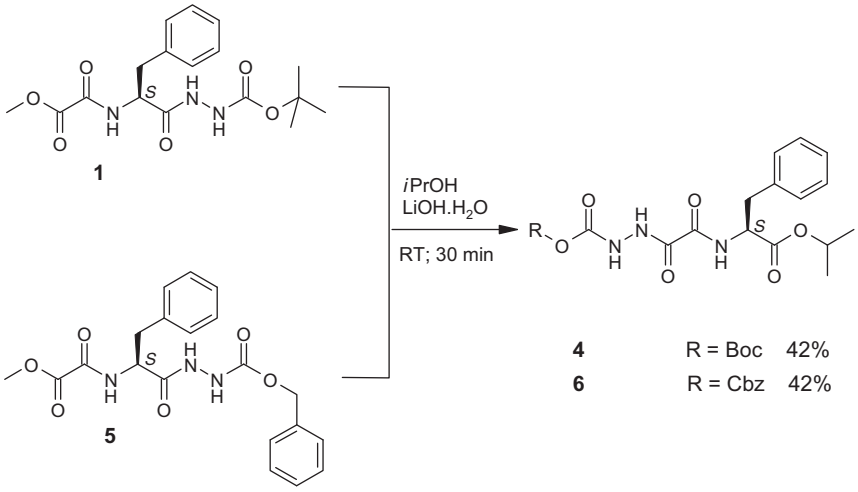


Scheme 3. One pot synthesis of NXO-compounds.[4]

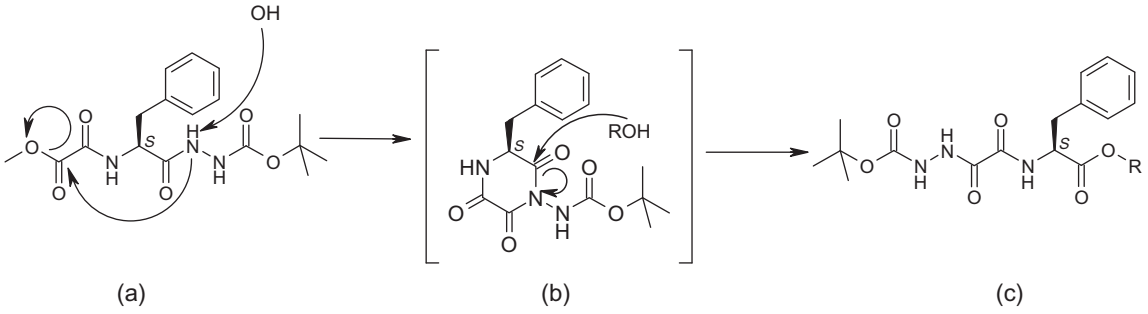
confirmed by NMR analysis and also by an alternative synthetic route (Scheme 2).[6]

Initially, it was thought that this rearrangement was the result of an internal cyclization, but attempts to synthesize the proposed cyclized structure were unsuccessful (Fig. 3).

To investigate this unusual behavior, a series of experiments were designed and performed. Firstly, the influence of the alcohol used as the solvent was examined because it was not clear whether the alkoxy function in the newly formed ester in **2** originated from the solvent alcohol or from the alcohol liberated from the starting



Scheme 4. LiOH-triggered solvolytic rearrangement of NXO-compounds using isopropanol as solvent.



Scheme 5. Proposed mechanism for the rearrangement.

material. The second objective was to determine if there was any effect due to the protecting group used to protect the hydrazide part of the NXO-compound on the outcome of the reaction. NXO-compounds were prepared via a reported procedure[4] using L-phenylalanine (Scheme 3) with different protecting groups such as Boc (*tert*-butyloxycarbonyl) and Cbz (benzyloxycarbonyl) to protect the hydrazide end.

These NXO-compounds were subjected to methyl ester cleavage using lithium hydroxide as a mild base, but with different alcohols as the solvent. Synthesis of compound **2** was previously reported,[6] when ethanol and isopropanol were used as the solvents, these reactions resulted in compounds **3** and **4**. The results confirmed that the ester in the rearranged product was derived from the alcohol used as the solvent (Scheme 1).

NXO-compounds containing L-phenylalanine with different protecting groups were subjected to solvolysis. It was observed that both the Boc and Cbz compounds showed similar behavior toward lithium hydroxide, generating rearranged products **4** and **6** (Scheme 4) in comparable yields.

During hydrolysis of an NXO-compound using DMSO as an aprotic solvent, no rearrangement was observed, and methyl ester hydrolysis occurred exclusively.

A proposed mechanism for the rearrangement is depicted in Scheme 5. Initial deprotonation leading to the intramolecular ring-closure gives a reactive intermediate cyclic piperazine-trione (b) which subsequently ring-opens to give the rearranged product.

In conclusion, a new rearrangement process was observed in NXO-modified building blocks of L-phenylalanine, with the ester part in the final products from derived alcohol used as the solvent. Moreover, such rearrangement was not observed in the presence of an aprotic solvent. The nature of the protecting group did not affect the yield of the final products.[8]

Acknowledgments

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8. To a solution of **1** or **5** (1.00 g; 2.7 mmol) in solvent (MeOH/EtOH/*i*PrOH) (20 mL) was added $LiOH{\cdot}H_2O$ (0.115 g; 2.7 mmol) along with two drops of H_2O. The solution was stirred at room temperature for 15 min. The solvent was removed under reduced pressure and the residue was dissolved in H_2O and extracted with EtOAc (3×50 mL). The organic phase was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to give the rearranged product. This was recrystallized from CH_2Cl_2.
Ethyl *N*-{[2-(*tert*-butoxycarbonyl)hydrazino](oxo)acetyl}phenylalaninate (**3**):
White solid 0.48 g (51%); HPLC-purity 99%.
Mp 60–63 °C.
^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.11–1.42 (m, 12 H), 1.92–2.01 (m, 2H), 2.87–3.28 (m, 2H), 4.66–4.84 (m, 1H), 7.00–7.22 (m, 5H), 8.25 (br s, 1H), 8.76 (br s, 4H), 9.14 (br s, 1H).
^{13}C NMR (50 MHz, $CDCl_3$): δ = 14.1, 28.0, 37.5, 53.8, 60.5, 82.5, 127.1, 128.7, 129.3, 135.8, 155.9, 157.9, 160.4, 170.1. ESI-HRMS: *m*/*z* calcd for $C_{18}H_{25}N_3O_6Na$ $[M+Na]^+$ 402.1641. Found: 402.1642.
ESI-HRMS: *m*/*z* calcd for $C_{18}H_{25}N_3O_6Na$ $[M+Na]^+$ 402.1641; found: 402.1642.
Isopropyl *N*-{[2-(*tert* butoxycarbonyl)hydrazino] (oxo)acetyl}phenylalaninate (**4**):
White solid 0.40 g (42%); HPLC-purity 99%.
Mp 62–64 °C.
^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.24 (d, *J* = 6.5 Hz, 6H), 1.36 (s, 9H), 2.96–3.26 (m, 2H), 4.66–4.81 (m, 1H), 4.94–5.09 (m, 1H), 6.58 (br s, 1H), 7.10–7.27 (m, 5H), 7.74 (br s, 1H), 8.37 (br s, 1H).
^{13}C NMR (50 MHz, $CDCl_3$): δ = 21.4, 28.1, 37.5, 53.2, 71.8, 82.0, 127.1, 128.7, 129.3, 135.8, 155.2, 156.9, 159.2, 169.6.
ESI-HRMS: *m*/*z* calcd for $C_{19}H_{27}N_3O_6Na$ $[M+Na]^+$ 416.1798; found: 416.1797.
Isopropyl *N*-[{2-[(benzyloxy)carbonyl]hydrazino} (oxo)acetyl]phenylalaninate (**6**):
White solid 0.45 g (42%); HPLC-purity 96%.
Mp 53–54 °C.
^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.19 (dd, $J_{1,2}$ = 1.8 Hz and 6.3 Hz, 6H), 2.89–3.20 (m, 2H), 4.90–5.05 (m, 3H), 6.93 (br s, 1H), 7.11–7.31 (m, 10H), 7.75 (br s, 1H), 8.53 (br s, 1H).
^{13}C NMR (50 MHz, $CDCl_3$): δ = 16.2, 32.3, 48.0, 62.6, 66.6, 121.9, 122.9, 123.1, 123.3, 123.5, 124.1, 130.2, 130.4, 150.8, 151.8, 153.8, 164.7.
ESI-HRMS: *m*/*z* calcd for $C_{22}H_{25}N_3O_6Na$ $[M+Na]^+$ 450.1641; found: 450.1641.