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Surprise in the Lithium Hydroxide Hydrolysis of a *NXO*-Compound.

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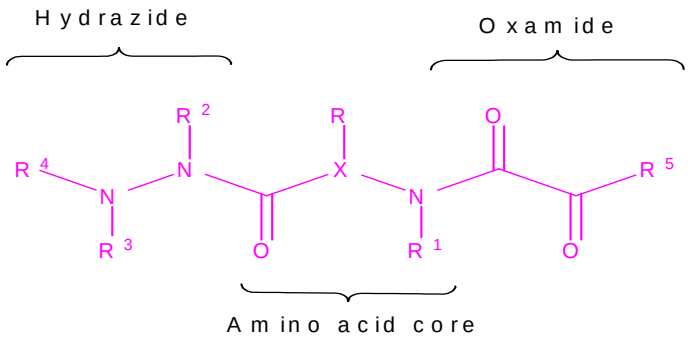
Abstract. This paper describes the unexpected outcome of the lithium hydroxide hydrolysis of the *NXO*-compound Boc-*NPheO*-OMe (**1**) and an independent synthesis of the product thus obtained, as well as the synthesis of 1,2,5 triazine-3,4,7- trione from **1**.

We submit these results for discussion to ECSOC 13 and hope to receive constructive comments and suggestions.

Introduction. Modified peptides are designed to mimic the biological function of natural peptides with modified stability, degradation or reactivity properties. Peptide mimics are prepared using modified amino acids or amino acid analogs. Earlier we have introduced modified amino acids[(1)] and have named them *NXO* compounds of the general formula **(A),** wherein X connects an acid hydrazide with an oxalic amide and additionally contains any residue of a natural or unnatural amino acid. These *NXO* compounds represent amino acid derivatives, where the basic amino group functionalized into an acidic oxamide group and the carboxylic acid is turned into an intrinsically basic hydrazide.

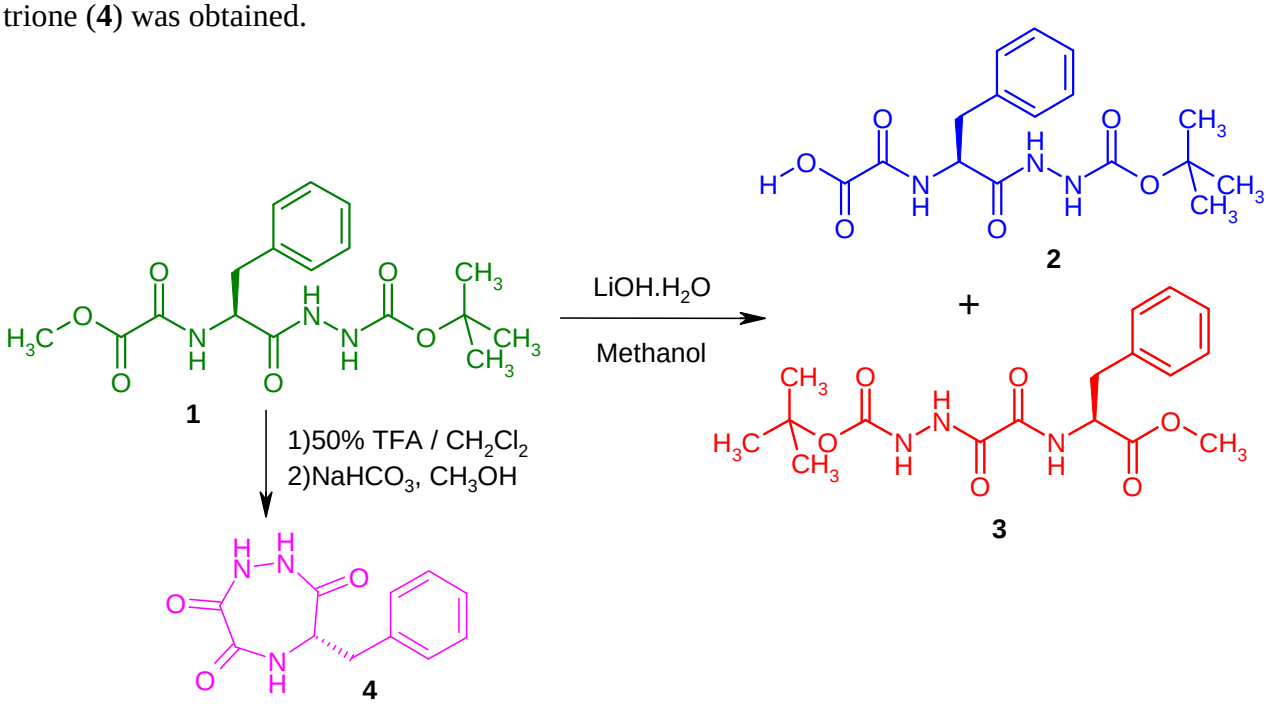
NXO-compounds (**A**)



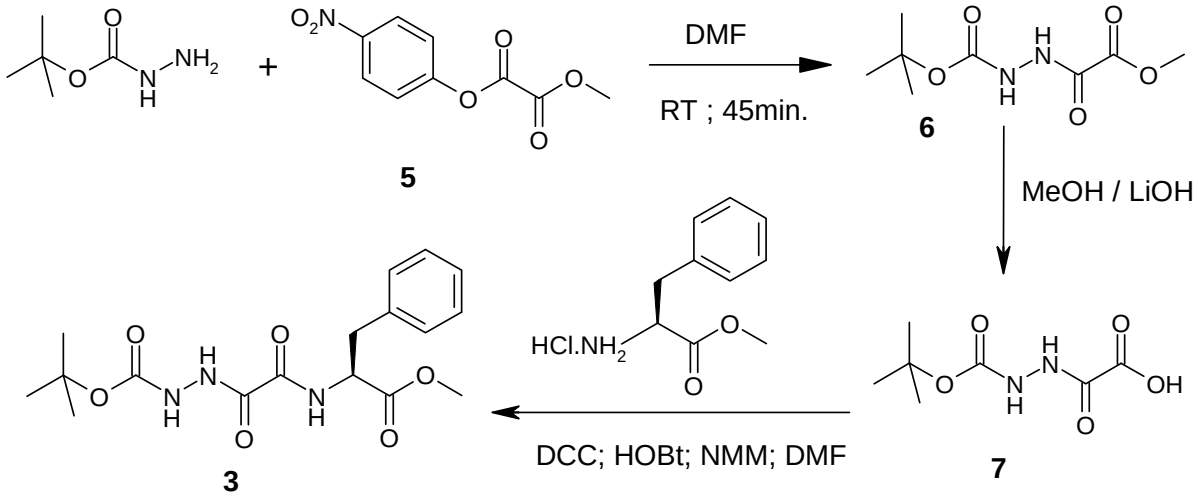
Methyl esters serve as protecting groups, which retain enough reactivity to be converted into amides or peptides. Their use for the protection of the carboxyl group is, however, rather limited as their removal, especially with a growing peptide chain usually is not efficient. The simple and most frequent used method for the hydrolysis of methyl ester is saponofication with aqueous alkali, mostly in the presence of varying amounts of organic solvents such as dioxane, methanol, ethanol, acetone, dimethylformamide etc.[2,3] . More specifically lithium hydroxide in methanol is used frequently for the hydrolysis of methyl esters in peptide chemistry.

Results and Discussion

While studying the chemical behaviour of the NXO-Compound derived from phenylalanine Boc-*NPheO*–OMe (**1**) we observed, in addition to the expected product **2,** another compound **3,** that was isolated and showed to have the same number of carbon peaks in 13C spectra to the starting material **1.** 2D NMR studies established the structure **3** for the isolated compound. The structure was further confirmed by an independent synthesis (*Scheme 1*). On treatment of **1** with TFA the novel dihydro-1,2,5-triazine-3,4,7-trione (**4**) was obtained.



The independent synthesis of **3** started from Boc-hydrazine. The intermediated **6** and **7** have not been reported before. DCC coupling of **7** with the Phe-OMe gave **3** while was identical in all respects with the compound obtained from **1.**



Scheme 1: Preparation of methyl*N*-[[2-(*tert*-butoxycarbonyl)hydrazino](oxo)acetyl]phenylalaninate**(3)**

Conclusion: In summary, we identified the structure of an unexpected product obtained from Boc-NPheO-OMe and developed an independent synthesis. We invite the scientific community to comment or explain this behaviour or suggest further experiments.

Experimental:

Reaction of Boc-NPheO-OMe with LiOH

To a solution of Boc-NPheO-OMe **1** (345mg; 94mmol) in 10 mL of methanol $LiOH.H_2O$ (39mg; 94mmol) was added and stirred at room temperature for 15min. The solvent was removed under vacuum; the remaining solid was dissolved in water and extracted with ethyl acetate. The organic phase was dried (Na_2SO_4) to give compound **3** Yield 58%. HPLC 98% 13C NMR 200MHz ($CDCl_3$) 28.07, 37.79, 52.55, 53.55, 82.34, 127.33, 128.74, 129.14, 135.24, 154.22, 157.71, 158.11, 170.60. The aqueous phase was adjusted to pH3 by dropwise addition of 5N HCl at 0°C. Extraction with ethyl acetate, washing of the organic phase with brine, drying using Na_2SO_4 and evaporation gave 130mg of **2**

(S)-6-Benzyl-dihydro-1,2,5 triazepane-3,4,7-trione (4)

[1]To the solution of **1** (1.0g, 2.74mmol.) in dichloromethane (15mL) TFA (15mL) was added drop-wise and the reaction mixture stirred at RT for 30 min. under argon. The reaction mixture was then evaporated and dried. The residue obtained was dissolved in dichloromethane (15mL) and washed with 10% $NaHCO_3$. The aqueous solution was extracted with dichloromethane (3 x 15mL), the combined organic layers were dried over Na_2SO_4 and evaporated under vacuum. The residue obtained was refluxed overnight in MeOH (15mL) under nitrogen. The solvent was evaporated and the crude compound obtained was purified by column chromatography to give 350mg (54%) of desired compound as a white solid. 1H NMR (200MHz, $CDCl_3$) (ppm) 8.3 (br s,1H), 7.1-7.4 (m, 5H), 6.7 (br s, 1H),4.7-4.9 (m, 1H), 3.04-3.36(m,2H). 13C NMR (200MHz, $CDCl_3$) (ppm) 171.83, 159.2, 158.6, 135.7, 129.23, 128.67, 127.1, 53.1, 37.5.

***tert*-Butyl 2-[methoxy(oxo)acetyl]hydrazinecarboxylate (6)**

To a solution of t-butylcarbazate (2.00g; 15mmol) in 20mL of DMF *tert*-butyl 4-nitrophenyl oxalate (3.75g; 17mmole was added and stirred under argon at room temperature for 45 min. 50mL of 10% $NaHCO_3$ was added and the reaction mixture was extracted with ethyl acetate and dried over Na_2SO_4. Ethyl acetate was removed in vacuum, to get the target compound **6.** Yield 25%; HPLC >93%; 13C NMR 200MHz ($CDCl_3$) 27.96, 53.60, 82.31, 154.69, 155.40, 159.56.

[2-(*tert*-Butoxycarbonyl)hydrazino](oxo)acetic acid (7)

A solution of **6** (830mg; 38mmol) and $LiOH.H_2O$ (160mg; 38mmol) in 15 mL of methanol and 2 drops of water were stirred at room temperature for 30min. Volatiles were removed under vacuum, the solid was dissolved in water and extracted three times with ethyl acetate. The aqueous phase was adjusted to pH3 by drop-wise addition of 5N HCl at 0°C. Extraction with ethyl acetate, washing of the organic phase with brine, drying using Na_2SO_4 and evaporation gave desired product in that was used as such in the next step. HPLC >99%; 13C NMR 200MHz ($CDCl_3$) 27.93, 79.45, 154.60, 158.17, 161.32.

Methyl-*N*-[[2-(*tert*-butoxycarbonyl)hydrazino](oxo)acetyl]phenylalaninate (3)

To a solution of **7** (120mg; 0.8mmol.) in 20mL of dichloromethane, HOBt (114mg; 0.84mmol) and DCC (173mg; 0.84mmol) were added and stirred under argon. Phenylalanine methyl ester hydrochloride (173mg; 0.8mmol) in 5mL of dichloromethane was treated with NMM (0.08mL), and the mixture was added to above solution followed by stirring under argon. Volatiles were removed under vacuum, the reaction mixture re-dissolved in ethyl acetate and cooled for 4 hours. This was filtered and the precipitated DCCU formed was washed with ethyl acetate. The combined organic phases were extracted with 1N HCl, 10% $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated to give **3** as colorless solid. Yield 26% ; HPLC >98% ; 13C NMR 200MHz ($CDCl_3$) 28.06, 37.74, 52.54, 53.55, 82.25, 127.30, 128.72, 129.14, 135.28, 154.28, 157.77, 158.17, 170.64.

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