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[a023]

Preparation of monoalkyl terephthalate and New photoactivatable probe to characterization of glutathione-binding proteins

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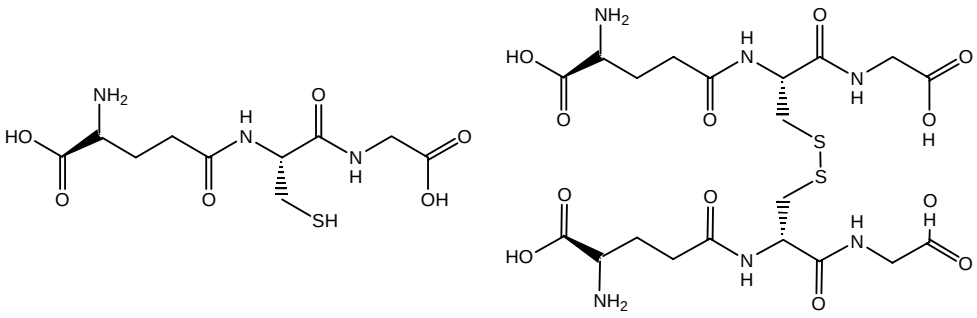
ABSTRACT

We described here the synthesis of a new photoactivatable probe to characterize glutathione-binding proteins. The preparation has been developed starting from toluene and monoalkyl terephthalate. We have shown that the latter can be easily obtained from terephthalic acid via a two steps procedure.

INTRODUCTION

Glutathione (Scheme 1) takes a predominant part in apoptosis (programmed cell death), a process which is deficient in many cancers. The action of glutathione is closely related to the activity of several proteins having a strong affinity for this tripeptide and which constitute the so-called glutathione system.

Scheme 1: The two forms of glutathione
a) on left, reduced glutathione GSH b) on right, oxidized glutathione GSSG.

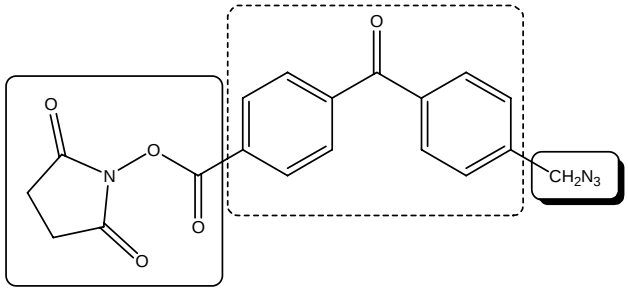


Therefore, we decided to identify these target proteins in order to facilitate the understanding of the relationship between the glutathione system and apoptosis in cancer cells.
Techniques of proteins labelling are often based on the use of radioactive compounds, which necessitate specific safety material and equipments and generate wastes that are more and more difficult and expensive to discard. Therefore, a radioisotope-free photoaffinity labeling[1] method was chosen for the identification of glutathione-binding proteins.

RESULTS AND DISCUSSION

We have carry out a new photoactivatable probe (Scheme 2) which can be connected to different biological molecules[2] including, in our case, reduced glutathione (GSH).

Scheme 2: New photoactivatable probe



Activated ester for GSH coupling

Photoactivable group able to covalently bind the target proteins

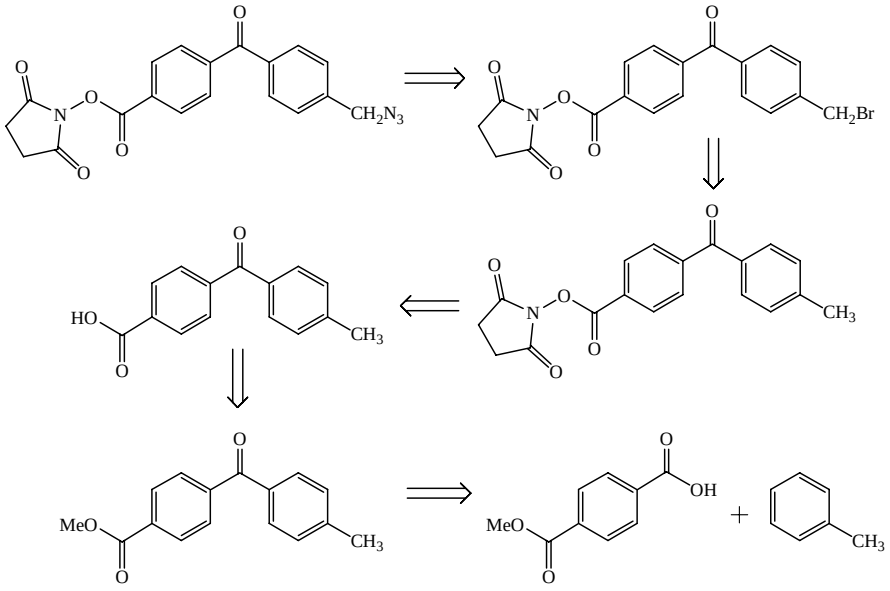
Group allowing the detection of captured proteins

Irradiation of a biological sample with this probe should lead to the formation of a covalent bond with proteins having a strong affinity for GSH. In a second step, the Staudinger-Bertozzi ligation[3] with a modified biotin should allow the visualization and identification of the target proteins and the binding sites. Indeed, the presence of biotin can be easily detected by a well known procedure using streptavidin-peroxydase and luminol and advantageously replaces the use of radioisotope.

EXPERIMENTAL

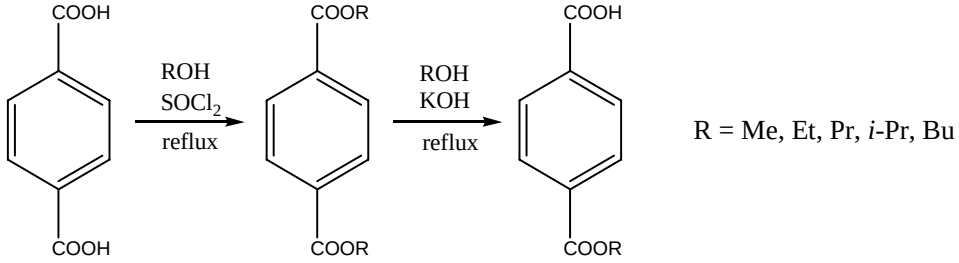
The scheme 3 represents one retrosynthetic way of this photactivatable probe. The azidomethylene group is generated in two steps from a methyl group. The active ester is obtained by saponification and reaction of the free acid on N-hydroxysuccinimide. The benzophenone moiety is formed via a classical Friedel-Crafts reaction.

Scheme 3



Monoalkyl terephthalate is the starting point of this synthetic way but it is quite expensive. Therefore, we have optimized a preparation method[4] from the very cheap terephthalic acid. (Scheme 4)

Scheme 4



CONCLUSION

A new photactivatable probe with a benzophenone framework has been synthesized. Identification of the proteins will be possible after glutathione coupling, irradiation and Staudinger-Bertozzi ligation and should allow a better understanding of their role in apoptotic process.
Syntheses of structurally modified photoaffinity probes are actually underway.

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