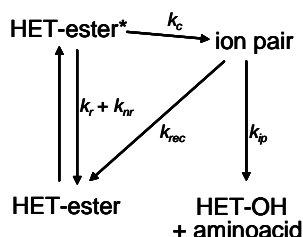


## Investigating photocleavage using time-resolved emission spectra

*The choice of protecting group is of crucial importance in the success of many steps in organic synthesis and the manipulation of polyfunctional molecules, since they can prevent the formation of undesired side products and reactions. Photoremovable protecting groups exhibit numerous advantages, such as the relatively soft conditions required for their deprotection and orthogonality with respect to acid- or base-sensitive groups. They have found application for the controlled release of a variety of molecules in the areas of materials science, the caging and release of biologically significant compounds and in synthetic organic chemistry.*

### Photocleavage reaction

The photocleavage reaction typically involves the formation of an ion pair from the excited ester, which can either recombine or split to form the corresponding alcohol, see simplified scheme for an amino acid- heterocycle system below



Scheme I. Simplified photocleavage scheme

It can then be the case that there will be a mixture of fluorescing species present. Potentially each will have a characteristic decay time and spectrum.

An objective is to attain control over the cleavage conditions; good photochemical yield, good cleavage rate and selectivity via choice of wavelength. Considering the wavelength it is advantageous for this to be longer than ~350nm as it can avoid, in the case of biological applications, cell damage caused by UV light. It offers the possibility of the use of two-photon excitation, which is advantageous for biological applications, and enables an improvement in selectivity, as only a small (femto litre) volume will be excited.

### Time-resolved fluorescence measurements

Since it is likely that the chosen system, upon photoexcitation, will contain several spectrally overlapping species, a simple steady state measurement will not be sufficient to characterise the system and elucidate the dynamics involved. The use of a time-resolved technique, such as measuring the time-resolved emission spectrum (TRES), is useful in obtaining this information. The TRES

acquisition is dependent on the presence of an emission monochromator. A system that can be used is the *HORIBA Scientific FluoroCube* shown below.

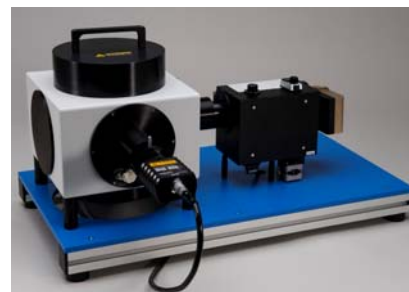


Fig. 1. FluoroCube-01 with DeltaDiode excitation

A typical measurement involves incrementing the monochromator in fixed wavelength steps, with time-resolved decays acquired for either fixed time intervals or to a predetermined peak count at each wavelength. To obtain intensity information, the option of fixed time interval should be chosen. Acquisition of the instrumental response (IRF or prompt) allows further analysis using reconvolution. It is also possible to obtain the decay associated spectra, shown schematically in Fig.2 – also see relevant *HS Technical Note*.

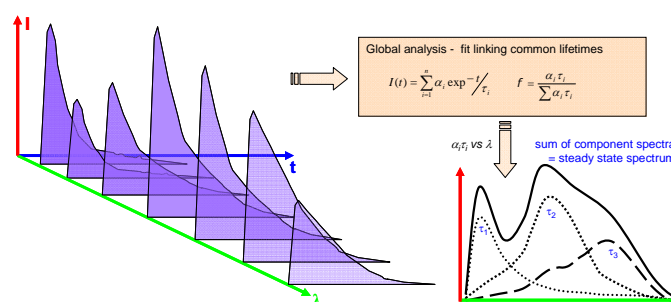
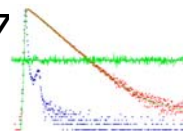


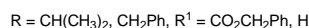
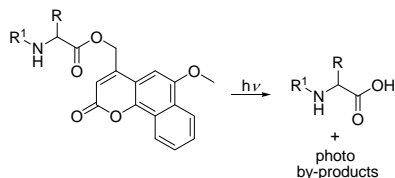
Fig. 2. Scheme for obtaining decay associated spectra

This enables spectra to be related to the different decay times and is helpful in elucidating the species present.



### Photocleavage of novel amino acid ester derivatives

This example is based on the work of *Costa* and *Gonçalves* (see reference) using compounds shown in Scheme II.



Scheme II. Compounds used and expected reaction

Time-resolved decays, measured using a *FluoroCube*, showed that the substituent moieties on the amino acid influenced the decay kinetics. This is demonstrated in the data shown in Fig. 2. This shows the effect of a substituent change on the time-resolved fluorescence behaviour. A model compound (to represent the photocleaved HET-OH moiety) was also measured. One of the advantages of time-resolved fluorescence measurements is that visually a qualitative interpretation of the data can be quickly made. From the top panel it is easily seen that the decay kinetics are different. DAS calculated from a TRES measurement are displayed in the other panels. Summing the individual DAS enables the overall (=steady state) spectrum to be obtained. These data allow comparisons, with model compounds for example, and assignment of species to be made. In the bottom panel it is apparent that the DAS for the longer lived decay is similar to the spectrum of the model compound.

Also from the lifetime values it is possible to estimate the rate constants shown in Scheme I. This gives a brief indication of the usefulness of time-resolved fluorescence techniques in this field of application.

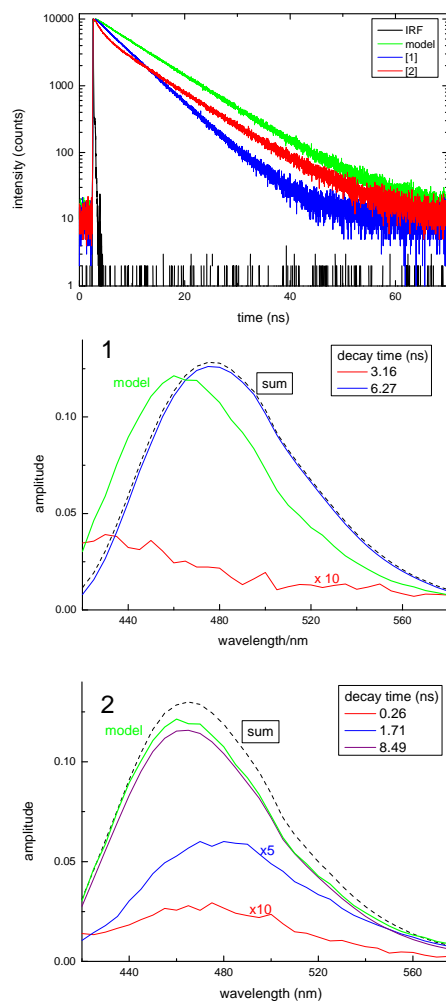


Fig. 2. Example data from time-resolved lifetime and DAS analysis of compounds shown in Scheme II

The results shown in this note are based on the following paper,

A.M. Piloto, A.M.S. Soares, G. Hungerford, S.P.G. Costa and M.S.T. Gonçalves, **2011**. *Photolysis at long wavelengths of amino acid ester derivatives based on 4-methyl-6-methoxy-2-oxo-2H-naphtho[1,2-b]pyrans*. Eur. J. Org. Chem. 5447-5451.

