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Research Article

Dansyl linked solid phase resins as an educational tool to teach the concepts of high throughput screening and prodrugs.

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ABSTRACT

High throughput screening (HTS) and combinatorial chemistry have been part of the process of drug design for nearly two decades. However, methods to teach their practical aspects to undergraduate students are scarce in the literature. The authors describe the synthesis and reactivity, under hydrolytic and transesterification conditions, of newly prepared fluorescent solid phase resins, envisaged to teach the concept of HTS, and prodrugs, to undergraduate students. Although the enzymatic methods did not perform well, the chemical hydrolysis, and the transesterification reaction, of these new solid phase resins, worked in very good yields; and produced very useful results. In relation to the experiment, the student's learning experience, and feedback, were very positive.

INTRODUCTION

The concept of high throughput screening (HTS) is described in many undergraduate medicinal chemistry textbooks (Silverman, 2004; Patrick, 2013; Wermuth, 2008) and at least one organic chemistry textbook (Patrick, 2014). Although research in HTS has had a big impact on biomedical research in the last 20 years (Macarron *et al.*, 2011), examples in the literature of an HTS experiment for compulsory undergraduate students' practicals are very scarce (Wetland *et al.*, 2004; Johnson R. J. *et al.*, 2015; Johnson A. J., 2015).

The concept of prodrugs, a compound that has to undergo a metabolic transformation before becoming an active pharmacological agent, it is also very important in medicinal chemistry and pharmacology. Given that about 10% of the drugs approved worldwide can be classified as prodrugs (Zawilska *et al.*, 2013), it is important to teach this concept to undergraduate students. Unfortunately, hands-on laboratory experiments involving chemicals, to teach this concept, are also scarce (Lavaggi *et al.*, 2013).

The authors have developed an easy, inexpensive, and fast practical to teach the concepts of high throughput screening, HTS, and prodrugs.

When combinatorial chemistry and HTS are used for research purposes, a library of compounds is prepared and tested by means of costly automated processes (Wermuth, 2008), which are not helpful for educational purposes. Therefore, we decided to avoid the automated processes, and show the students a manual approximation, using a new experiment that is affordable within the undergraduate laboratories' budget.

Hence, we developed an experiment that uses fluorescence to visualize a "hit". Those hits can be visualised just by utilising the common UV lamp, used to visualize thin layer chromatography (TLC) plaques. However, for quantification, which adds to the experiment, a Fluoroskan® apparatus is recommended.

Student feedback was analysed for internal purposes and was never intended to constitute an evaluation of

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teaching methods of any kind. Hence, the number of students participating was considered adequate for our purposes.

MATERIALS AND METHODS

We initially thought about using lipases for this experiment, not only because lipases cleave esters, and esters are the most common prodrugs used (Rautio *et al.*, 2008); but also, because lipases are the most frequently used enzymes in organic synthesis (Singh *et al.*, 2016). They are easy to handle, relatively inexpensive, and do not require a cofactor, which simplifies the experiments. Furthermore, lipase inhibitors are easily accessible molecules through mainstream chemical companies.

Although there are methods for HTS of lipase activities already described, which can be considered straightforward in the hands of experienced personnel (Reymond, 2004), these methods are time-consuming, and require a level of experience that undergraduate students generally lack.

To provide a simple method, for educational purposes, we decided to use a substrate, marked with a fluorescent probe, and linked to a solid support. For the choice of solid support, we chose the most applicable support for enzyme based-chemistry (Kress *et al.*, 2002) : cross-linked 2-acrylamidoprop1-yl[2-aminoprop-1-yl]polyethylene glycol, PEGA-based resin, in particular amino methyl PEGA resin, **1**. This type of resin provides an extensive and uniform swelling in a wide range of solvents, including water (H_2O) and ethanol (EtOH) (Camarero *et al.*, 1998), and has the unique feature of allowing on-resin enzymatic assays (Meldal *et al.*, 1994). The fact that it has been used in combination with lipase enzymes (Humphrey *et al.*, 2003), confirmed to us that this resin was appropriate for our intended application.

General experimental details.

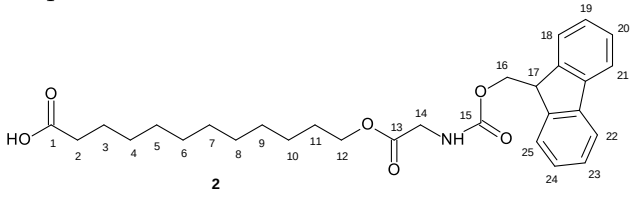
The graphics used in this manuscript were created using Microsoft Excel. Pictures of the resins are fluorescent wells, created using a Samsung J3 mobile under a UV lamp. The modifications of the original pictures, when needed, were done with the Microsoft Paint program. The heating of the samples was performed by means of a Grant BTD Dry Block heating block, and the sample centrifugation by means of a Hettich Mikro 20 Microcentrifuge. The fluorimetry experiments were performed by means of a Fluoroskan® Ascent FL apparatus with an excitation/emission filter pair of 390 / 518 nm. The infrared absorbances were recorded on a Perkin Elmer Spectrum 3 FT-IR Spectrometer L1280133. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained on a Bruker DPX400 spectrometer.

Organic synthesis

*Calculation of the loading of PEG resin **1**.*

Commercial amino methyl PEGA resin **1**, which is wet in EtOH for stability (3.00 g, equivalent approximately to 240 mg of dry resin, 0.096 mmol) was placed in a solid phase vessel. The resin was dried inside a desiccator under vacuum, swelled dichloromethane (CH_2Cl_2) (8 mL) for 5 min, dried, washed with dimethylformamide (DMF) (3 x 3 mL x 3 min) and was left suspended in DMF (3 mL). In a separate vial, a mixture of *N,N*-diisopropylethylamine DIPEA (466 mg, 3.60 mmol), Fmoc-Gly-OH (892 mg, 3.00 mmol) and O-(1H-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU) (1197 mg, 2.89 mmol) was prepared in DMF (3 mL), and the mixture was allowed to activate for 3 min. This mixture was added to the suspended resin, and coupling was allowed to proceed by mechanical agitation (150 oscillations per minute, o.p.m.) for 18 h. After that procedure, the Kaiser test was still positive for free amines. The procedure was repeated twice more until the Kaiser test was negative.

The wet resin was washed with CH_2Cl_2 (2 x 3 mL x 3 min) and dry it under vacuum. 7 mg of the resin were treated with 1 mL of 2% solution of 1,8-Diazabicyclo [5.4.0] undec-7-ene (DBU) in DMF for 30 min. The solution was placed in a volumetric flask and topped up with acetonitrile up to 5 mL. A 1 mL portion of this was placed in 25 mL volumetric flask and top up with acetonitrile. A blank was made with the same concentration and procedure. Absorption of the resin and the blank was measured at 304 nm. After the calculations, the loading of the resin was 0.4 mmol/g. This was considered as the maximum loading that any compound attached to the PEGA resin **1** could have.



2

*Synthesis of Fmoc-Glycine lauric acid ester, **2**.*

In a round bottom flask, Fmoc-Glycine (1189 mg, 4.00 mmol), HCTU (827 mg, 2.00 mmol) and DIPEA (322 mg, 2.49 mmol), were dissolved in DMF (4 mL) and, left stirring for 5 min to be activated. Over the mixture, 12-hydroxylauric acid (216 mg, 1.00 mmol), dissolved in DMF (1.5 mL), was added drop by drop for 2 min and, the reaction was left stirring for 3 h. Water (50 mL) was added to obtain a white precipitate. The precipitate was filtered, washed with water (150 mL) and centrifuged (2000 rpm) for 5 min. The solid was collected and dissolved in ethyl acetate (EtOAc) (150

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mL). The organic solution was washed with brine (2 x 20 mL), dried ($MgSO_4$), filtered and evaporated to obtain a white waxy solid crude (1.53 g). The crude was purified by chromatography over silica gel; after purification eluting with petroleum ether / EtOAc 75:25 and, posterior purification with CH_2Cl_2 / AcEOt 90:10, compound **2** was obtained pure as a white crystal (271 mg, 58%).

IR (cm^{-1}) 3311.66 (OH st), 2924.17, 2851.49, 1763.46 (C=O st), 1688.29 (C=0 st), 1535.06.
^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10-1.60 (m, 14H, CH_2-4, CH_2-5, CH_2-6, CH_2-7, CH_2-8, CH_2-9 and CH_2-10), 1.54 (m, 4H, CH_2-3 and CH_2-11), 2.25 (t, 2H, CH_2-2), 3.91 (d, $J \approx 6.0$ Hz, 1H, H-14a), 4.07 (dd, $J \approx J' \approx 6.0$ Hz, 1H, H-14b), 4.15 (t, $J = 7.1$ Hz, 1H, CH-17), 4.32 (d, 2H, $J = 7.1$ Hz, H-16), 5.35 (t, $J \approx J' \approx 6.0$ Hz, 1H, -NH-), 7.21 (ddd, $J \approx J' \approx 7.0$ Hz, $J'' = 1.1$ Hz, 2H, H-19 and H-24), 7.31 (ddd, $J = J' = 7.4$ Hz, $J'' = 0.3$ Hz, 2H, H-20 and H-23), 7.52 (d, $J = 7.4$ Hz, 2H, H-18 and H-25), 7.67 (d, $J \approx 7.4$ Hz, 2H, H-21 and H-22).
^{13}C NMR (100 MHz, $CDCl_3$): δ 24.71, 25.80, 28.53, 29.04, 29.18, 29.21, 29.25, 29.43, 29.50, 34.05, 42.81, 47.12, 65.76, 67.27, 120.01, 125.13, 127.10, 127.74, 141.31, 143.84, 156.40, 170.27, 179.27.
MS: *m/z* calculated for $C_{29}H_{37}NO_6$: 495.26; found: 496.31 $[M+H]^+$, 519.32 $[M+Na]^+$

Synthesis of fluorescent resin ***4***.
Commercial amino methyl PEGA resin **1**, which is wet in EtOH for stability (625 mg, equivalent approximately to 50 mg of dry resin, 0.02 mmol as resin load is 0.4 mmol/g) was placed in a solid phase vessel. The resin was swelled in CH_2Cl_2 (2 mL) for 5 min, dried, washed with DMF (3 x 2 mL x 3 min) and was left suspended in DMF (2 mL). The suspension was treated with DIPEA (13 mg, 0. 1 mmol), 4-Dimethylaminopyridine (DMAP) (2 mg, catalytic) and dansyl chloride (27 mg, 0.1 mmol) and the mixture was left shaking (250 o.p.m.) at room temperature for 18 h. The resulting resin was washed twice with each of the following sequence of solvents: DMF, CH_2Cl_2, diethyl ether (Et_2O), CH_2Cl_2 and DMF (2 mL x 3 min) and then treated twice with a solution of Ac_2O (0.5 M) and DIPEA (0.5 M) in DMF (2 mL) for 5 min each time. The resulting resin was washed again twice with the following sequence of solvents: DMF, CH_2Cl_2, Et_2O, CH_2Cl_2 (2 mL x 3 min). The resin was washed with Et_2O (1 mL) and dried. The resulting solid resin (54 mg) was left wet in EtOH (1 mL) at 4 °C to ensure stability.

Synthesis of fluorescent resin ***3***.
Commercial amino methyl PEGA resin **1**, which is wet in EtOH for stability (2.50 g, equivalent approximately to 200 mg of dry resin, 0.08 mmol) was placed in a solid phase vessel. The resin was swelled in CH_2Cl_2 (8 mL) for 5 min, dried, washed with DMF three times (3 mL x 3 min each wash) and was left suspended in DMF (1 mL). In a separate vial, DIPEA (22 mg, 0.170 mmol) was added to compound **5** (67 mg, 0.135 mmol), 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo [4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) (49 mg, 0.129 mmol) was also added dissolved in DMF (1 mL), and the mixture was allowed to activate for 3 min. This mixture was added to the suspended resin and coupling was allowed to proceed by mechanical agitation (150 o.p.m.) for 24 h. The reaction mixture was filtered, washed with a sequence of DMF, CH_2Cl_2, MeOH, and Et_2O (3 mL x 3 min x 2) and then treated with a solution of acetic anhydride (Ac_2O) (0.5 M) and DIPEA (0.5 M) in DMF (1 mL x 5 min x 2). The acetylating reagents were removed by vacuum suction and the resin was washed with a sequence of DMF, CH_2Cl_2, MeOH, CH_2Cl_2 and EtOH (3 mL x 3 min x 2) and was left wet in EtOH. The loading of the resin was calculated by treatment with a 2% solution of DBU in DMF and the resulting loading was 0.3 mmol/g.

This wet resin was dried under a stream of N_2 to eliminate the EtOH, then washed with CH_2Cl_2 (5 mL x 5 min x 3) and dry under vacuum. The dry resin was washed with DMF (5 mL x 5 min x 2) and treated with a 2:2:96 mixture of piperidine/DBU/DMF (5 mL x 5 min x 4). The resulting resin was washed with each of the following sequence of solvents: DMF, CH_2Cl_2, MeOH, Et_2O, CH_2Cl_2 (3 mL x 3 min x 2) and was left wet in EtOH.

The wet resin was dried under a stream of N_2 to eliminate the EtOH, then washed three times with CH_2Cl_2 (5 mL x 5 min) and dry under vacuum. This resin (246 mg, theoretically 0.074 mmol) was placed in a solid phase vessel, washed with DMF twice (5 mL x 5 min), filtered, and suspended in DMF (6 mL). The suspension was treated with DIEA (48 mg, 0. 37 mmol), DMAP (8 mg, catalytic), and dansyl chloride (100 mg, 0.37 mmol) and the mixture was left shaking (200 o.p.m.) at room temperature for 21 h. The resulting resin was washed with each of the following sequence of solvents: DMF, CH_2Cl_2, Et_2O, CH_2Cl_2 and DMF (5 mL x 3 min x 2) and then treated (5 min x 2) with a solution of Ac_2O (0.5 M) and DIPEA (0.5 M) in DMF (2 mL). The acetylating reagents were removed by vacuum suction and the resulting resin was then washed with the following sequence of solvents: DMF, CH_2Cl_2, Et_2O, CH_2Cl_2 (5 mL x 3 min x 2) and dried under high vacuum for 6 h to produce 171 mg of resin, which was left wet in EtOH (3 mL) at 4 °C for better stability. Using the above-described conditions, we calculated the loading of the resin to be 0.3 mmol/g.

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Synthesis of fluorescent resin ***5***
Wang resin (100 mg, 0.75 mmol/g, 0.075 mmol) was placed in a solid phase vessel. It was swelled in CH_2Cl_2 (2 mL) for 5 min. The resin was washed with DMF (2 mL x 3 min x 3) and the resulting resin was then treated with a solution of DIPEA (24 mg, 0.185 mmol), DMAP (4 mg, 0.033 mmol), dansyl chloride (51 mg, 0.185 mmol) in DMF (2 mL), and the mixture was left shaking (200 o.p.m.) for 18 h. After that, the resin was filtered, and washed with a succession of solvents DMF, CH_2Cl_2, Et_2O, CH_2Cl_2, DMF (all 3 mL x 2 min x 2). Although the resin was fluorescent when freshly prepared, it became non-florescent overnight, despite being kept at 4 ^oC.

Synthesis of fluorescent resin ***6***
Rink amide MBHA resin (100 mg, 0.4-0.8 mmol/g, 0.04 to 0.08 mmol) was placed in a solid phase vessel. It was swelled in CH_2Cl_2 (2 mL) for 5 min. The resin was washed with DMF (2 mL x 3 min x 3) and it was treated with a mixture of piperidine/DBU/DMF 2:2:96 (5 mL x 5 min x 4). After that, the resin was filtered, and washed with a succession of solvents CH_2Cl_2, MeOH, Et_2O, CH_2Cl_2, DMF (all 3 mL x 3 min x 2).
The resulting resin was then treated with a solution of DIPEA (52 mg, 0.40 mmol), DMAP (8 mg, 0.067 mmol), dansyl chloride (108 mg, 0.40 mmol) in DMF (3 mL), and the mixture was left shaking for 18 h. After that, the resin was filtered, washed with a succession of solvents DMF, CH_2Cl_2, Et_2O, CH_2Cl_2, DMF (all 3 mL x 2 min x 2) and analysed by IR. The rest of the resin was treated with a solution of Ac_2O / DIPEA 0.5 M/0.5 M (3 mL x 3 min x 3). The acetylating reagents were removed by vacuum suction, washed with a succession of solvents DMF, CH_2Cl_2. Et_2O. CH_2Cl_2 (all 3 mL x 2 min x 3) and dried in a desiccator to obtain a yellow resin (110 mg after discounting the resin taken for IR analysis).
IR (cm^{-1}) 2924.35, 1655.08 (C=O st), 1667.42 (C=O st), 1293.12 (S=O st).

Synthesis of fluorescent resin ***7:***
In a solid phase vessel, a solution of leucine (21 mg, 0.158 mmol) and DIPEA (82 mg, 0.632 mmol) in a mixture of CH_2Cl_2 (2 mL) and DMF (0.5 mL), was prepared. Over the solution, trityl chloride resin (1.6 mmol/g, 0.158 mmol, 100 mg) was added and the mixture was left shaking for 18 h. After that, the resin was filtered, and washed with a mixture of CH_2Cl_2/MeOH/DIPEA 17:2:1 (5 mL x 3 min x 4), then CH_2Cl_2 / DMF / and CH_2Cl_2 (all 5 mL x 1 min x 2). The resulting resin was then treated with a solution of DIPEA (103 mg, 0.797 mmol), DMAP (16 mg, 0.13 mmol), dansyl chloride (208 mg, 0.772 mmol) in DMF (4 mL), and the mixture was left shaking for 20 h. After that, the resin was filtered, and washed with a succession of solvents DMF, CH_2Cl_2. Et_2O. CH_2Cl_2, DMF (all 4 mL x 1 min x 2) and analysed by IR.
IR (cm^{-1}) 2924.12, 1744.92 (C=O st), 1335.66 (S=O st).

Synthesis of fluorescent resin ***8***
Commercial amino methyl PEGA resin **1**, which is wet in EtOH for stability (3.00 g, equivalent approximately to 240 mg of dry resin, 0.096 mmol) was placed in a solid phase vessel. The resin was dried inside a desiccator under vacuum, swelled in CH_2Cl_2 (8 mL) for 5 min, dried, washed with DMF (3 x 3 mL x 3 min),and left suspended in DMF (3 mL). In a separate vial, a mixture of DIPEA (466 mg, 3.60 mmol), Fmoc-Gly-OH (892 mg, 3.00 mmol) and HCTU (1197 mg, 2.89 mmol) was prepared in DMF (3 mL), and the mixture was allowed to activate for 3 min. This mixture was added to the suspended resin, and coupling was allowed to procced by mechanical agitation (150 o.p.m.) for 18 h. After that procedure, the Kaiser test was still positive for free amines. The procedure was repeated twice more, until the Kaiser test was negative.

The wet resin was washed with CH_2Cl_2 (3 mL x 3 min x 2) and dried under vacuum. 7 mg of the resin were treated with 1 mL of 2% solution of DBU in DMF for 30 min. The solution was placed in a volumetric flask and topped up with acetonitrile to 5 mL. A 1 mL portion of this was placed in 25 mL volumetric flask and top up with acetonitrile. A blank was made with the same concentration and procedure. Absorption of the resin and the blank were measured at 304 nm. After the calculations, the loading of the resin was 0.4 mmol/g. This was considered the maximum loading that any compound attached to the PEGA resin **1** could have.

Part of this Fmoc-Gly-resin (330 mg, 0.4 mmol/g, 0.132 mmol) was treated with DMF (2 mL) for prewash, and then treated with piperidine / DMF 1:4 (3 mL x 5 min x 3). It was washed with DMF (5 mL x 5 min), DIPEA/DMF 1:3 mixture (4 mL x 5 min), and then DMF (5 mL x 5 min x 2). The resulting resin was washed again with the following sequence of solvents: CH_2Cl_2, MeOH, CH_2Cl_2, DMF (5 mL x 5 min x 2). The resulting resin was then treated with a solution of DIPEA (26 mg, 0.20 mmol), DMAP (8 mg, 0.032 mmol), dansyl chloride (56 mg, 0.20 mmol) in DMF (4 mL), and the mixture was left shaking for 18 h. After that, the resin was filtered, washed with a succession of solvents DMF, CH_2Cl_2, Et_2O, CH_2Cl_2, DMF (all 2 mL x 2 min x 2), and then treated twice with a solution of Ac_2O (0.5 M) and DIPEA (0.5 M) in DMF (2 mL x 5 min). The acetylating reagents were removed by vacuum suction; the resulting resin was washed again with the following sequence of solvents: DMF, CH_2Cl_2, Et_2O, CH_2Cl_2 (2 mL x 3 min x

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2). The resin was washed with Et_2O (1 mL) and dried. The resulting solid resin (354 mg) was analysed by IR, and showed a little bit of free amine, and the rest was left wet in EtOH (1 mL) at 4 ºC to ensure stability.
IR (cm^{-1}) 3482.79 (NH st), 2869.79, 1624.23 (C=O st), 1348.86 (S=O st).

Synthesis of trityl lauric resin ***12.***
In a solid phase vessel, a solution of Fmoc-Glycine lauric acid ester (**2**, 56 mg, 0.114 mmol) and DIPEA (59 mg, 0.456 mmol) in a mixture of CH_2Cl_2 (2 mL) and DMF (0.5 mL), was prepared. Over the solution, trityl chloride resin (1.6 mmol/g, 0.114 mmol, 72 mg) was added and the mixture was left shaking for 18 h. After that, the resin was filtered, washed with a mixture of CH_2Cl_2/MeOH/DIPEA 17:2:1 (5 mL x 3 min x 4), then CH_2Cl_2, DMF, and CH_2Cl_2 (all 5 mL x 1 min x 2). The resin was dried in a desiccator and once dried, was treated with a mixture of piperidine/DBU/DMF 2:2:96 (3.5 mL x 5 min x 3). After that, the resin was filtered, washed with a succession of solvents DMF, CH_2Cl_2, Et_2O, CH_2Cl_2, DMF (all 3 mL x 1 min x 2).
IR (cm^{-1}) 2924.05, 2852.94, 1739.00 (C=O st), 1707.87 (C=O st), 1677.38 (C=0 st).

The resulting resin was then treated with a solution of DIPEA (74 mg, 0.572 mmol), DMAP (12 mg, 0.1 mmol), and dansyl chloride (150 mg, 0.556 mmol) in DMF (4 mL), and the mixture was left shaking for 20 h. After that, the resin was filtered, washed with a succession of solvents DMF, CH_2Cl_2, Et_2O, CH_2Cl_2, DMF (all 4 mL x 1 min x 2) and analysed by IR.
IR (cm^{-1}) 2924.12, 1744.92 (C=O st), 1667.42 (C=O st), 1335.66 (S=0 st).

The rest of the resin was treated with a solution of Ac_2O / DIPEA 0.5 M/0.5 M (5 mL x 5 min x 2). The acetylating reagents were removed by vacuum suction, washed with a succession of solvents DMF, CH_2Cl_2, Et_2O, CH_2Cl_2 (all 4 mL x 1 min x 2) and dried in a desiccator to obtain a yellow resin (94 mg after discounting the resin taken for IR and loading analysis).

Procedure for the practical with LiOH
General fluorescent resin experiment.
This experiment was done in triplicate. In a 1 mL Eppendorf tube, a suspension of LiOH 0.1 N solution in MeOH (100 µL), dioxane (100 µL) and any of the fluorescent resins **3, 8, 6, 4** or **7** (approximately 2 mg) were prepared. The suspension was incubated for 30 min at 37 ºC, shaking the tube after 15 min. DMF (100 µL) was added and the precipitate was centrifuged (2700 rpm for 2 min). The supernatant was separated and this showed marked fluorescence under an ordinary UV lamp. 100 µL of this supernatant were added to a 96 black well plate and the fluorescent values were recorded by means of a Fluoroskan® Ascent FL apparatus with an excitation/emission filter pair of 390 / 518 nm.

The supernatants were analysed by LC-MS and the peak of dansyl glycine only, was detected by MS (*m/z* calculated for $C_{14}H_{16}N_2O_4S$: 308.08; found: 309.23 $[M+H]^+$).

General blank experiment with the resins.
In a 1 mL Eppendorf tube, a suspension of MeOH (100 µL), dioxane (100 µL), and any of the fluorescent resins **3, 8, 6, 4** or **7** (approximately 2 mg) was prepared. The suspension was incubated for 30 min at 37 ºC, shaking the tube after 15 min. DMF (100 µL) was added and the precipitated was centrifuged (2700 rpm for 2 min). The supernatant was separated, and this showed marked fluorescence under an ordinary UV lamp. 100 µL of this supernatant were added to a 96 black well plate and the fluorescent values recorded by means of a Fluoroskan® Ascent FL apparatus with an excitation/emission filter pair of 390 / 518 nm. Because of the small fluorescent values obtained, and the value of the fluorescent resins, we did not see any need of recording this experiment in triplicate.

General blank experiment with the resins.
In a 96 black well plate, a mixture of MeOH / dioxane 1:1 (100 µL) was added, and the fluorescent values recorded by means of a Fluoroskan® Ascent FL apparatus with an excitation/emission filter pair of 390 / 518 nm. Because of the even smaller fluorescent values obtained, we did not see any need of recording this experiment in triplicate.
The fluorescent values, obtained during the LiOH reaction are depicted in Supplemetary (Supp.) Materials.

Procedure for the practical with NaOMe
General fluorescent resin experiment.
This experiment was done in triplicate. In a 1 mL Eppendorf tube, a suspension of NaOMe 0.01 N solution in MeOH (100 µL), dioxane (100 µL) and any of the fluorescent resins **3, 8, 6, 4** or **7** (approximately 2 mg) was prepared. The suspension was incubated for 30 min at 37 ºC, shaking the tube after 15 min. DMF (100 µL) was added and the precipitated was centrifuged (2700 rpm for 2 min). The supernatant was separated and this showed marked fluorescence under an ordinary UV lamp. 100 µL of this supernatant were added to a 96 black well plate and the fluorescent values recorded by means of a Fluoroskan® Ascent FL apparatus with an excitation/emission filter pair of 390 / 518 nm.
The supernatants were analysed by LC-MS and the peak of dansyl glycine methyl ester, was mainly

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detected by MS (*m/z* calculated for $C_{15}H_{18}N_2O_4S$: 322.10; found: 323.23 $[M+H]^+$).

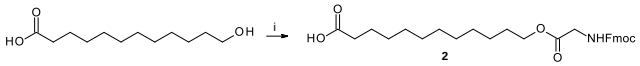
The fluorescent values, obtained during the NaOMe reaction are depicted in Supp. Materials.

Hydrolysis of Trityl resin Lauric ester
In a 1 mL Eppendorf tube, a suspension of NaOMe 0.01 N solution in MeOH (100 µL), dioxane (100 µL) and the fluorescent resins **12** (2 mg) was prepared. The suspension was incubated for 30 min at 37 ºC, shaking the tube after 15 min. DMF (100 µL) was added and the precipitate was centrifuged (2700 rpm for 2 min). The supernatants were analysed by LC-MS and the peak of compound **13**, was mainly detected by MS unexpectedly (*m/z* calculated for $C_{27}H_{40}N_2O_6S$: 520.26; found: 521.28 $[M+H]^+$).

General blank experiment with the resins.
In a 1 mL Eppendorf tube, a suspension of MeOH (100 µL), dioxane (100 µL) and any of the fluorescent resins **3, 8, 6, 4** or **7** (approximately 2 mg) was prepared. The suspension was incubated for 30 min at 37 ºC, shaking the tube after 15 min. DMF (100 µL) was added and the precipitate was centrifuged (2700 rpm for 2 min). The supernatant was separated and this showed marked fluorescence under an ordinary UV lamp. 100 µL of this supernatant was added to a 96 black well plate and the fluorescent values were recorded by means of a Fluoroskan® Ascent FL apparatus with an excitation/emission filter pair of 390 / 518 nm. Because of the small fluorescent values obtained, and the value of the fluorescent resins, we did not see any need of recording this experiment in triplicate.

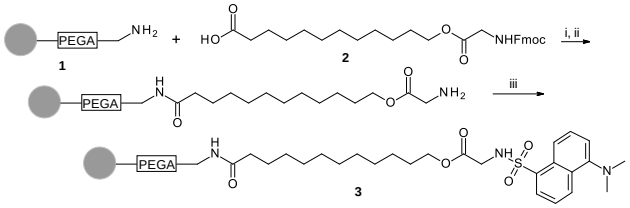
General blank experiment with the resins.
In a 96 black well plate, a mixture of MeOH / dioxane 1:1 (100 µL) was added, and the fluorescent values recorded by means of a Fluoroskan® Ascent FL apparatus with an excitation/emission filter pair of 390 / 518 nm. Because of the even smaller fluorescent values obtained, we did not see any need of recording this experiment in triplicate.

Therefore, our target was to synthesize a fluorescent substrate that should be easily cleaved by hydrolytic enzymes such as lipases, esterases, or proteases. We thought this fluorescent molecule should be composed of PEGA-based solid support, linked by lauric acid to a dansyl molecule as the fluorescent marker. To prepare it, solution-phase and solid-phase synthetic techniques were used in the synthesis. This started with compound **2**, as a linker, synthesised from Fmoc-glycine and 12-hydroxylauric acid, as seen in Scheme 1.



Scheme 1. Synthesis of lauric acid Fmoc glycine **2.** i) HCTU/DIPEA/ Fmoc glycine in DMF.

The first fluorescent resin (compound **3**), was synthesized as shown in Scheme 2, with 75% yield approximately, calculated by comparing the values of Fmoc cleavage with the resin loading, and assuming an almost quantitative yield in the last step.

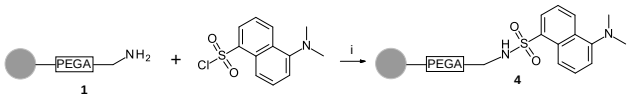


Scheme 2. Attachment of **2** to PEGA resin, **1**, and transformation into lauric glycine dansyl fluorescent PEGA resin, **3**. i) **2**, HATU/DIPEA/DMF; ii) DBU/Piperidine/DMF; iii) Dansyl chloride, DIPEA/ DMAP/DMF

We also decided to prepare fluorescent resin **4**, in which the dansyl marker was linked directly to the resin (Scheme 3). This fluorescent resin could act as a reference, because the sulphonamide bond should not be cleaved by lipases, esterases, or proteases.

When a small molecule such as dansyl chloride is directly linked to the PEGA resin, we can expect higher loading and a maximum fluorescence value *per* weight ratio, and this was the case.

It is important to note that all these resins (**1**, **3** and **4**) should be kept in EtOH, as the beads of PEGA resins become very sticky and easily damaged when shrunk or dry (Novabiochem catalogue, 2014).



Scheme 3. Synthesis of reference resin **4.** i) DIPEA/DMAP/DMF

The fluorescent resin **3** as it can be seen in figure 1, produced an intense fluorescence, when suspended in CH_2Cl_2 and visualized under the conventional UV lamp at 356 nm.

After the hydrolysis of resin **3**, in the time conditions of the assays, the fluorescence values are expected to come only from dansyl glycine.

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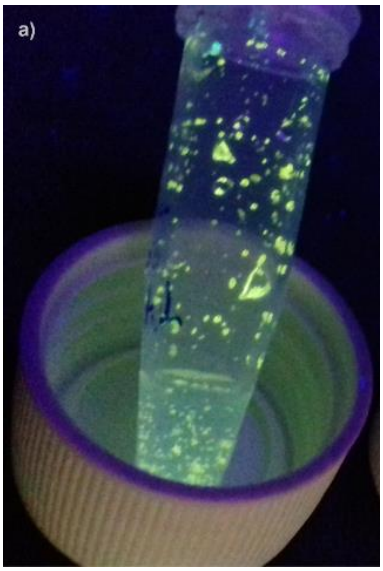

Figure 1. Clearly visible fluorescence under the UV lamp (365 nm) of resin **3**.

Thus, before starting the enzyme assays, a calibration curve was plotted for the commercially available dansyl glycine (Figure 2), after optimising the instrument's settings. The fluorescence signals were best detected using the excitation/emission wavelength of 390 / 518nm.

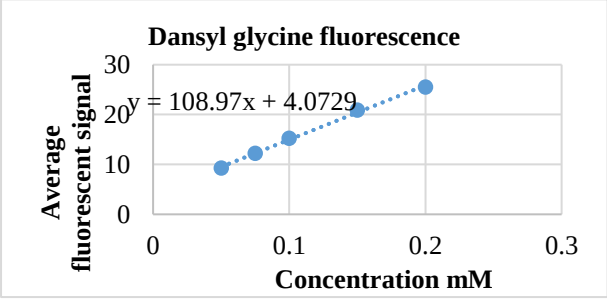

Figure 2. Fluorometric calibration curve of the commercially available dansyl glycine. The fluorescence values are expressed in relative fluorescence units (RFU).

The next step was the enzymatic hydrolysis using tris(hydroxymethyl) aminomethane (TRIS) buffer at pH = 8, which has been successfully applied before (Campanile *et al.*, 2016). The purpose of this step was to get fluorescence values related to the amount of dansyl glycine released into the solution. Therefore, we designed a new method that required precipitation of the enzyme and subsequent centrifugation. This method avoids any residue of the suspended resins, which will have noticeable fluorescent activity.

Unfortunately, all trials conducted using hog pancreas lipase, with resin **3** inside Tris hydrochloride (HCl) buffer (pH=8) in the different conditions described (Campanile *et al.*, 2016; Yang et al., 1990; DiNella *et al.*, 1960), did not show significant fluorescence values, using the Fluoroskan® instrument. This was surprising since traces of dansyl glycine were detected, by means of liquid chromatography-mass spectrometry (LC-MS). In practice, the Fluoroskan® values were close to the values for the blank (*i.e.* non marked resin + lipase suspension + TRIS buffer), and this was not sufficiently differentiated for our purpose.

Lipase reactions using an aqueous buffer with an added small percentage of dimethyl formamide (DMF), have been used successfully in many publications (Kumar *et al.*, 2016; Kamal *et al.*, 2013) and they do not produce enzyme denaturation or activity change (Kamal *et al.*, 2013).[23] In our case, adding DMF will swell the resin and could facilitate enzyme penetration. In addition, adding $CaCl_2$ has also been described to increase enzymatic activity (Horchani *et al.*, 2009; Kimura *et al.*, 1982; Naka & Nakamura, 1994). However, adding either DMF, or $CaCl_2$ did not produce a real improvement in the fluorescent values. This was surprising because we designed resin **3** with a long hydrocarbon chain, which could, in theory, bring our molecule closer to the surface to react with the enzyme, minimizing the effect that the enzyme molecular size could have.

It is worth noting that PEGA resin derivatives have been successfully hydrolyzed by lipases, but the best results were not from hog pancreas lipase but *Chromobacterium viscosum* lipase (*C. viscosum* lipase) (Laurent *et al.*, 2008; Humphrey *et al.*, 2003). The commercial PEGA resins are only partially accessible for enzymes with a molecular weight of up to 35-40 KDa (Kress *et al.*, 2002). Although many experimental procedures described in the literature, involving lipases, do not clearly state the molecular weight of the enzymes used; an article that describes the purification of two closely related porcine pancreas lipases, found that the molecular weight (MW) was 45-50 kDa (Verger *et al.*, 1969). Thus, the molecular weight aspect may have been the most important factor, taking into consideration that the lipase from *C. viscosum* lipase has a MW of 33 KDa (Taipa *et al.*, 1995).

Finally, we were able to obtain the desired product, clean, by treatment with LiOH 0.1 N in MeOH/Dioxane (Dayal *et al.* 1990), at 37 °C. After precipitation of the resin and enzyme with DMF, and subsequent centrifugation, the resulting supernatant, was a marked fluorescent solution. Considering the loading of resin, the synthetic scheme, the hydrolysis of the dansyl linked moiety, and the comparison with the calibration curve early depicted, the reaction happened in nearly quantitative yield. This ester

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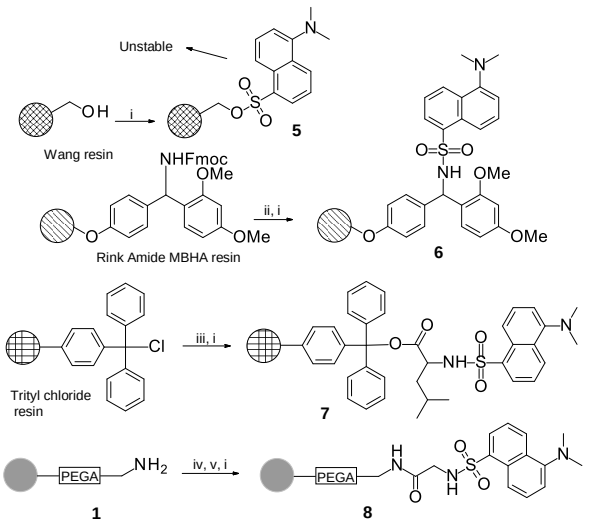
hydrolysis is a good model to explain ester hydrolysis, of prodrugs, inside the body. The only small drawback of this reaction is that we saw an unexpected hydrolysis of resin **8** (table 2.1 supplementary material) which is described later and contains an amide. That could confuse the students.

A transesterification reaction, in which the dansyl moiety is cleaved leaving a fluorescent ester instead of the acid, was tried too. Therefore, sodium methoxide (NaOMe) 0.01 N in MeOH (Ohtani *et al.*, 1991) was performed with great yield.

Because our main objective was to prepare a practical experiment, we needed more than one fluorescent resins. With this idea in mind, we prepared several fluorescent dansyl-linked resins. Those fluorescent resins that were, *a priori*, not susceptible to react, will be used as negative controls. The chosen resins were dansyl linked Wang resin **5**, dansyl linked rink amide MBHA resin **6**, dansyl leucine trityl resin **7** and dansyl glycine linked PEGA resin **8** (scheme 4). Together, with resins **3** and **4**, we had enough compounds for a small library.



Scheme 4. Variety of fluorescent resins prepared for the undergraduate laboratory practical. i) Dansyl chloride, DIPEA / DMAP / DMF. ii) Piperidine /DBU/DMF 2:2:96. iii) 9-Fluorenylmethoxycarbonyl protected (Fmoc) Leucine, DIPEA. iv) Fmoc-glycine, DIPEA / HCTU / DMF. v) 2% DBU in DMF.

RESULTS AND DISCUSSION

Once we had the resins and the methodology for cleaving them, we thought that we could first show the students a group of fluorescent resins, under the UV lamp. Then we could ask the students to weigh them and perform an experiment. At the end of the experiment, they should have a fluorescent supernatant in some resins but not in others. The reactivity differences between the resins should prove the concept we wanted to teach.

The undergraduate experiment that we are describing now, will teach transesterification reactions, which is the one that we decided to use. But could be used to teach hydrolysis too, if you are not focusing on ester hydrolysis only, because the LiOH method can also hydrolise amides. Which resin can be used for each experiment can be deduced from section 2 of the Supp. Materials.

The experiment envisaged, will show a positive signal in the 96 well plate, the equivalent of a 'hit'. This 'hit' will not only be visible under the UV lamp, but also could be quantified, by means of a Fluoroskan® apparatus, just in case there was any doubt.

The designed practical was tried and evaluated by three undergraduate chemistry students. The participating students gave very positive feedback, as described later. We collected feedback from participating students, to show that this is a feasible undergraduate practical.

Description of the undergraduate practical

Before the experiment, a questionnaire was handed out to the students, to check their base knowledge of some subjects related to the practical experiment. The questionnaire contained 23 questions with 3 types of possible answers: "I agree", "I disagree" and "I do not know". It is depicted in Figure 3, and included in Supp. Materials, at the end of this article. The correct answers to the questions were not given to the students at any time. Another questionnaire, with the same questions, but in a different order, was administered after the experiment.

The practical procedure was then handed out to the students, which contained an introduction to combinatorial chemistry and HTS. The steps of the chemical procedure were written on the handout, and each student had a micropipette and sufficient disposable tips. They had access to the fourth resins **3**, **4**, **6** and **8**, which were fluorescent under the UV lamp (Figure 4). All the resins where named ICT, plus a three letters code that was easy for them to remember and identify each resin.

In this respect, resin **3** was called ICT-CFL resin, resin **4** was called ICT-NHL resin, resin **6** was called the ICT-NBA resin, and finally, resin **8** was the ICT-MLS resin. Although all these resins contained the same fluorophore linked by a sulphonamide bond: the emission colour of each resin, under the UV lamp, is slightly different, as it can be seen in Figure 4. This may be related to the overall environment of the fluorophore inside the different resins.

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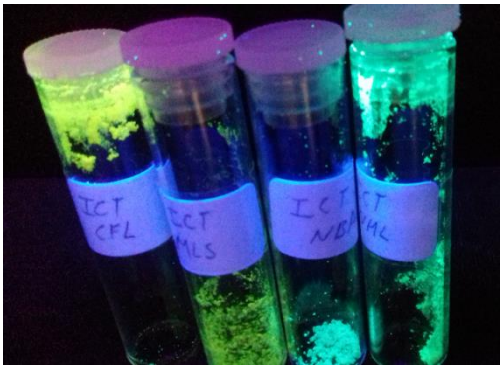

Figure 4. Resins available for the practical under the UV lamp (365 nm), all named ICT plus a 3 letter code.

Experimental procedure of the practical

The students had to weigh approximately 2 mg of each resin and placed it into a 1 mL Eppendorf vessel. They had to suspend the resin in dioxane, add a 0.01 M NaOMe in MeOH solution (freshly prepared) and heat the resulting suspension at 37 ºC for 1 h, as described in the experimental procedure. After the centrifugation of each sample, the supernatant was placed in a 96 well black plate, resistant to conditions used in the reactions. The plate was then visualized under the UV lamp (365 nm) as seen in Figure 5.

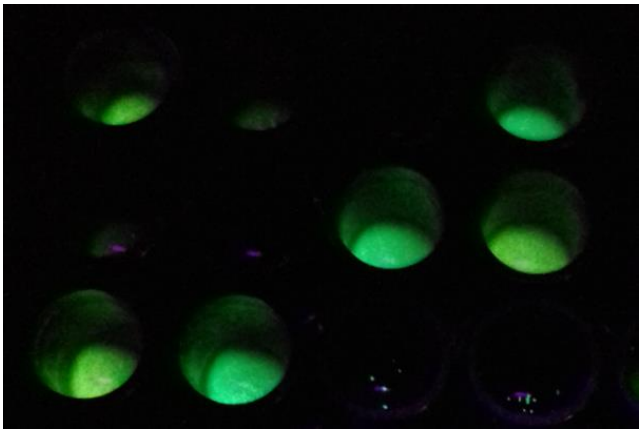

Figure 5. 96 well plate results of the student practical, preliminary experiment. Each row constitutes the experimental result of one student.

As can be seen in Figure 5, only two of the four plates, for each individual student's experiment, showed a potential "hit", which we previously defined as a supernatant that shows florescence. One was more clearly visible than the other, although it is not easy to see this in the picture. To dissipate any doubt, the demonstrator accompanied the students to measure the relative fluorescence units using a Fluoroskan® apparatus.. The fact that there was a clear positive hit, a doubtfully positive hit, and two negative hits also added to the student's learning experience.

The students' visual results are depicted in Figure 5 and, were similar to the ones initially described by the authors of this article (picture not shown). This demonstrates the reproducibility of this experiment in students' hands.

As previously mentioned, the students were given another knowledge questionnaire, after the experiment, which contained the same questions as before, but in a different order (Supp. Materials).

The comparison of data collected from questionnaires, administered before and after an undergraduate practical, is a method that has been described in the literature, as a mean to evaluate the teaching efficacy of a laboratory experiment (Campanile *et al.*, 2016; Morral *et al.*; 2015).

After comparing the responses from the two knowledge questionnaires, we found that the practical was helpful to students, in learning about combinatorial chemistry, as well as medicinal chemistry in general.

In this respect, this group of three undergraduate (third year) students demonstrated progress in relation to the material learnt, as we will describe later. They learnt new principles and expanded their knowledge through this practical experiment. We can then certify the utility of the practical, as a tool for learning about combinatorial chemistry and HTS. This experiment can also be used to teach about the design of prodrugs, since many prodrugs are esters that need to be hydrolysed to release the active drug (Rautio *et al.*, 2008).

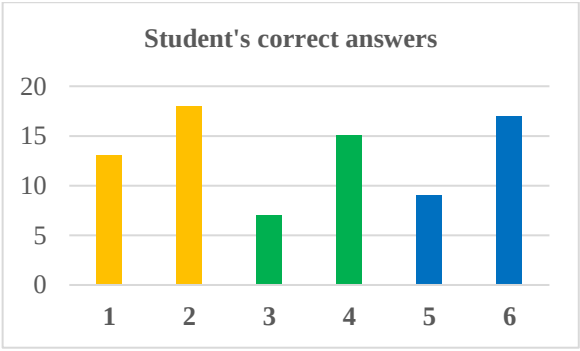

Figure 7. Representation of the results of the knowledge questionnaire, each colour represents one of the three students. Odd numbers represent the number of questions answered correctly before the practical. Even numbers represent the number of questions answered correctly after the students have completed the practical.

After the students filled out the questionnaire, they were also given an evaluation questionnaire to assess the learning experience itself (Supp. Materials).

The evaluation questionnaire indicated very positive feedback from the students, not only in relation to the knowledge acquired during the practical, but also in

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terms of overall satisfaction (Figure 8). As can be seen in Figure 8, the most frequent responses to the question statements were "strongly agree" (blue) or "agree" (orange). Furthermore, all the students were satisfied with the practical overall (Question 10).

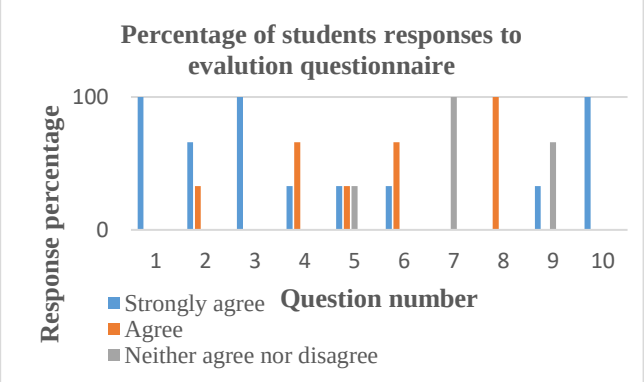

Figure 8. Depiction of the students' responses to the evaluation questionnaire, expressed as percentages.

NOTES FOR INSTRUCTORS

If the course coordinator prefers, the experiment can easily be conducted without using the Fluoroskan®. In this case, it is recommended to use only the LiOH procedure, to avoid using resin **8**; and to use resins **4**, **6**, and **7** alongside resin **3**. Because none of the resins, except resin **3**, will show fluorescence under the UV lamp, this experiment is perfectly feasible, and students can observe the fluorescence and understand the concept of a "hit".

Hazards

Although there are no toxicity studies on the resins, the nature of them (*e.g.* non-volatile, used in very small quantities, etc.) makes them potentially less dangerous than other chemicals used in undergraduate laboratory experiments. However, we recommend that the resins are treated in the same way as any other chemical. We obliged our students to follow laboratory safety procedures (laboratory coat, goggles, and gloves) when handling the chemicals and the resins. NaOMe is strongly alkaline, and we recommend the solution in MeOH to be freshly prepared by the demonstrator, or technicians, and then given to the students, ready to be used.

Some of the resins tend to stick to the spatula and glass. Although the weighed resins can be given to the students, we recommend allowing extra time so that the students weigh them. In this way, students will be in contact with a sticky chemical, not very common in undergraduate experiments, which will add to the experience. We also recommend weighing the resins by difference; this means from a vial containing more than the amount needed, and weighing the amount that is left in the vial. In this way, the small amount of resin stuck to the spatula can be deposited inside the Eppendorf using the solvent used in the reaction.

Although all the students can use freely the UV lamp without problems, if the experiments need the Fluoroskan®, or similar apparatus, they should be done by the demonstrator, or under supervision. A black 96 well plate, which allows for the recording of many experiments at once, and which is relatively resistant to solvents and bases, was used. Some transparent 96 well plates were not resistant to the organic solvents and bases used in the experiment, thus they are not recommended. If the Fluoroskan® is set up for other well plate sizes, they can also be used. However, they have to allow the recording of many experiments at once, to avoid delaying the experiment. With the Fluoroskan®, the experiment can be performed in a relatively short time, even with large groups, of up to 60 students. However, this practical experiment was designed to suit most chemical laboratories, even if they do not have a fluorimetry apparatus. In this described experiment, the Fluoroskan® is just an optional experiment to confirm the results by fluorimetry, as noted before.

All the resins must be kept in the fridge at <10 °C. When PEGA derivative resins are used, they must be kept wet in EtOH, thus the demonstrator must dry them before the practical, as described in the experimental procedure.

Classroom settings

This experiment was performed by three Chemistry third-year undergraduate project students and one demonstrator. The experiment was set up for a session of less than 3 h, including administering the questionnaires. When the laboratory practical becomes routine, feedback questionnaires will not be needed. Thus, even with the use of the Fluoroskan®, this practical should take far less than 3 h, independently of the size of the group.

In our set up, each student was working individually, and while the practical can easily be done in pairs, more than two people per experiment is not recommended. The chemistry laboratory was equipped with chemical fume hoods, and this is the recommended setup. A minimum of two demonstrators is compulsory, with one demonstrator performing the Fluoroskan® experiments. If the experiment is done without the final Fluoroskan® measuring, the practical will also be shorter, but a second demonstrator is still recommendable, particularly with a large group of students.

Each student had access to a 200 mL micropipette, and while in a larger classroom set up just 2 micropipettes

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could be used per bench, more is recommended. There were containers with soapy water in the fume hood to dispose of the used tips, which will contain traces of alkaline solution. High-accuracy balances were set up in a corner of the laboratory, and students had to go there to weigh the resins.

The incubator was kept in a separate room inside the chemistry laboratory, but it can be kept in the same chemistry laboratory, preferably inside a fume hood. Because our laboratory does not have a centrifuge, we used the centrifuge situated in the biology laboratory. A demonstrator carried the samples to the biology laboratory at intervals for centrifugation, then returned them to the chemistry laboratory for analysis of supernatants. A centrifuge can easily be placed in the laboratory to speed up the process, but it does not need to be inside the fume hood.

The Fluoroskan® machine was situated in a separate laboratory. The demonstrator accompanied the students, carrying the samples, to show them how it worked. For larger groups, we would recommend one black 96 dell plate for each student's bench (that can easily be used by 8 groups or more). In this way, students will put the samples in every well, using a pipette in an orderly fashion, and record the wells used for all the samples in their laboratory notebook. It is important to remember to use two wells as a blank for each resin: one for the resin without the reagent and one for the reagents and solvents on its own.
After the measuring, the demonstrator had to return to the chemistry laboratory to give the data to the students.

CONCLUSIONS

We have synthesized a new fluorescent PEGA resin, as well as other fluorescent resins, all using dansyl as a fluorescence marker. These resins can be used to teach HTS experiments to students, and the ester prodrugs concept, by means of two straightforward experiments that we have developed. One experiment is the hydrolysis with LiOH and another is the transesterification with NaOMe.

Both experiments can also be used to teach students concepts of combinatorial chemistry; as well as hydrolysis, transesterification reactions, and fluorimetry. The procedures described are fast and relatively easy. They include only relatively inexpensive reagents and the use of a small centrifuge. If the experiments are to be quantified by a Fluoroskan®, or the resins are used to teach fluorimetry, this easy-to-follow described procedure can also be used.

The assessment of the knowledge acquired from performing the practical, was by means of questionnaires administered before and after the practical. Student satisfaction with the practical was assessed by means of a questionnaire completed after the practical. The practical produced a marked increase in knowledge and the overall satisfaction with the practical was very positive.

The authors recommend using this laboratory experiment, or a modification of it, to teach undergraduate chemistry students.

ACKNOWLEDGEMENTS

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SUPPLEMENTARY MATERIALS

1. Students' Questionnaires

1.1. Copy of the knowledge questionnaire handed out to the undergraduate students, before the practical.

Practical questionnaire (before)

	I Agree	I disagree	I do not know
A chemical library is a family of compounds having a certain base chemical structure			
High-throughput screening are very rapid and sensitive *in vitro* screens			
High-throughput screening allows a large number of compounds, to be screened with just small amounts			
High-throughput screening is part of the drug-discovery process widely used in the pharmaceutical industry			
The information obtained in high-throughput screening is always unquestionable			
To date, high-throughput screening does not leverage automation to quickly assay the biological or biochemical activity of a large number of drug-like compounds, but can be possible in the future			
High-throughput screening is always linked to combinatorial chemistry			
Combinatorial chemistry is the synthesis or biosynthesis of *chemical libraries* of molecules for the purpose of biological screening, particularly for lead discovery or lead modification			
A lead compound is a compound that elicit a predetermined level of activity in the bioassay			
A hit is an active principle isolated from a natural source or a synthetic compound prepared in the laboratory.			
Prodrugs are pharmacologically active but their metabolites are not.			
A considerable proportion of the drugs approved worldwide (around 5%) are prodrugs			
Carboxylic esters are seldom used as prodrugs			
Carboxylic esters prodrugs are activated in the body by enzymatic hydrolysis (e.g. lipases and esterases)			
Carboxylic esters as prodrugs are most often used to enhance the lipophilicity, and thus the passive membrane permeability			
Phosphate ester prodrugs are typically designed for hydroxyl and amine functionalities of poorly water-soluble drugs because they enhance their aqueous solubility			
Phosphate ester prodrugs have good chemical stability and rapid bioconversion back to the parent drug by lipases and esterases			
Solid-phase synthesis is a method in which molecules are bound on a bead and synthesized step-by-step in a reactant solution and allows a relatively easy removal of the excess reactant or by-product from the product			
Combinatorial chemistry is often linked to solid phase			
Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation			
In relation to fluorescence, in most cases, the emitted light has a shorter wavelength, and therefore higher energy, than the absorbed radiation.			
We can always check the hydrolysis of an ester by means of fluorescence			
Being able to check the proportion of an ester prodrug that has been hydrolysed, is essential when developing esters prodrugs			

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1.2. Copy of the knowledge questionnaire handed out to the undergraduate students, after the practical.

Practical questionnaire (after)

	I Agree	I disagree	I do not know
A chemical library is a family of compounds having a certain base chemical structure			
Combinatorial chemistry is the synthesis or biosynthesis of *chemical libraries* of molecules for the purpose of biological screening, particularly for lead discovery or lead modification			
High-throughput screening are very rapid and sensitive *in vitro* screens			
High-throughput screening allows a large number of compounds, to be screened with just small amounts			
High-throughput screening is part of the drug-discovery process widely used in the pharmaceutical industry			
To date, high-throughput screening does not leverage automation to quickly assay the biological or biochemical activity of a large number of drug-like compounds, but can be possible in the future			
High-throughput screening is always linked to combinatorial chemistry			
A lead compound is a compound that elicit a predetermined level of activity in the bioassay			
A hit is an active principle isolated from a natural source or a synthetic compound prepared in the laboratory			
The information obtained in high-throughput screening is always unquestionable			
Prodrugs are pharmacologically active but their metabolites are not			
A considerable proportion of the drugs approved worldwide (around 5%) are prodrugs			
Carboxylic esters are seldom used as prodrugs			
Carboxylic esters prodrugs are activated in the body by enzymatic hydrolysis (e.g. lipases and esterases)			
Carboxylic esters as prodrugs are most often used to enhance the lipophilicity, and thus the passive membrane permeability			
Phosphate ester prodrugs are typically designed for hydroxyl and amine functionalities of poorly water-soluble drugs because they enhance their aqueous solubility			
Phosphate ester prodrugs have good chemical stability and rapid bioconversion back to the parent drug by lipases and esterases			
Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation			
In relation to fluorescence, in most cases, the emitted light has a shorter wavelength, and therefore higher energy, than the absorbed radiation			
We can always check the hydrolysis of an ester by means of fluorescence			
Being able to check the proportion of an ester prodrug that has been hydrolysed, is essential when developing esters prodrugs			
Solid-phase synthesis is a method in which molecules are bound on a bead and synthesized step-by-step in a reactant solution and allows a relatively easy removal of the excess reactant or byproduct from the product			
Combinatorial chemistry is often linked to solid phase			

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1.3. Copy of the evaluation questionnaire handed out to the undergraduate students after the HTS practical.

	Strongly agree	**Agree**	**Neither agree nor disagree**	**Disagree**	**Strongly disagree**
1) This practical has allowed me to learn more about combinatorial chemistry					
2) This practical has allowed me to learn more about solid phase synthesis					
3) This practical has allowed me to learn more about high-throughput screening					
4) This practical has allowed me to learn more about the drug discovery process					
5) This practical has allowed me to learn more about prodrugs					
6) This practical has allowed me to learn more about esters as prodrugs					
7) This practical has allowed me to learn more about fluorescence					
8) This practical has allowed me to learn more about medicinal chemistry in general					
9) This practical has allowed me to learn more about how student laboratory experiments are developed					
10) Overall I am satisfied with this practical					

Notes:

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2. Fluorescent values, obtained during the different reactions.

2.1. Fluorescent values, LiOH reaction

Resin	Fluorescence Values	Fluorescence Values	Fluorescence Values	mg of resin	mg of resin	mg of resin	Value/mg	Value/mg	Value/mg	Average/mg
3	415.7	466	420.4	2.05	2.19	1.93	2.027.805	2.127.854	2.178.238	2.111.299
8	495.4	454.6	416.4	2.03	2.26	1.88	2.440.394	2.011.504	2.214.894	2.222.264
6	0.752	0.5303	4.1	2.02	2.26	2.04	0.372277	0.234646	2.009.804	0.872242
4	17.04	77.82	19.48	2.03	2.12	2.16	8.394.089	3.670.755	9.018.519	1.804.005
7	2.027	2.185	2.253	2.01	2.02	2.05	1.008.458	1.081.683	1.099.024	1.063.055

Resin	Fluorescence Values Resin Alone	mg resin alone	Value/mg	Dioxane / MeOH	Value - resin - blank /mg	Value - resin - blank /mg	Value - resin - blank /mg	Average	Stand. Dev.
3	3.202	2.07	1.449.275	0.356	2.009.752	2.109.801	2.160.186	2.093.246	7.657.091
8	3.108	1.92	15.625	0.356	2.421.209	1.992.319	2.195.709	2.203.079	2.145.398
6	0.2639	2.18	1.376.147	0.356	-135.987	-14.975	0.277657	-0.8599	0.987558
4	5.845	2.22	1.351.351	0.356	6.686.737	350.002	7.311.167	163.327	1.616.954
7	1.937	1.98	1.515.152	0.356	-0.86269	-0.78947	-0.77213	-0.8081	0.048071

2.2. Fluorescent values, NaOMe reaction

Resin	Fluorescence Values	Fluorescence Values	Fluorescence Values	mg of resin	mg of resin	mg of resin	Value/mg	Value/mg	Value/mg	Average/mg
3	503.1	640.1	611.4	2.1	2.2	2.28	2.395.714	2.909.545	2.681.579	266.228
8	79.51	131.8	101.9	2.08	2.34	2.27	3.822.596	5.632.479	4.488.987	4.648.021
6	2.289	2.295	2.583	2.02	2.21	2.02	1.133.168	1.038.462	1.278.713	1.150.114
4	2.93	6.202	5.004	2.2	1.98	2.01	1.331.818	3.132.323	2.489.552	2.317.898
7	735.6	992.9	934.8	2	1.86	2.02	367.8	5.338.172	4.627.723	4.547.965

Resin	Fluorescence Values Resin Alone	mg resin alone	Value/mg	Dioxane / MeOH	Value - resin - blank /mg	Value - resin - blank /mg	Value - resin - blank /mg	Average	Stand. Dev.
3	2.019	2.07	0.975362	1.054	2.375.421	2.889.252	2.661.285	2.641.986	2.574.587
8	2.481	1.92	1.292.188	1.054	3.587.977	539.786	4.254.368	4.413.402	9.153.619
6	0.9306	2.18	0.426881	1.054	-0.34771	-0.44242	-0.20217	-0.33077	0.121019
4	1.715	2.22	0.772523	1.054	-0.4947	1.305.801	0.66303	0.491375	0.912444
7	752.4	1.98	380	1.054	-13.254	1.527.632	8.171.828	7.374.249	8.329.549