Reactivity Assays of Surface Hydroxyl Chain Ends of Poly(ethylene terephthalate) (PET) Film and Membranes Using Original ^{3}H- and Fluorine-Labeled Derivatization Reagents

Patrick Mougenot and Jacqueline Marchand-Brynaert*

Université catholique de Louvain, Laboratoire de Chimie Organique de Synthèse, Bâtiment Lavoisier, 1 place Louis Pasteur, B-1348 Louvain-La-Neuve, Belgium

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ABSTRACT: The hydroxyl chain ends (PET-OH) displayed on the surface of poly(ethylene terephthalate) film and track-etched membranes of two different porosities were assayed by derivatization with reagents containing ^{3}H and fluorine tags. After activation by tosylation, the resulting sulfonate esters (PET-OTs) were substituted with L-*N*-(trifluoroethyl)[4,5-^{3}H]lysinamide. On the other hand, direct coupling of PET-OH with heptafluorobutyl (*p*-isocyanatobenzoyl)[2-^{3}H]glycinate was performed; subsequent treatement with L-[4,5-^{3}H]lysine partially gave the ester substitution. All the samples were analyzed by liquid scintillation counting (LSC) and X-ray photoelectron spectroscopy (XPS). The ratios of derivatization recorded by LSC measurements, for membranes currently used as substrates in cell cultivation, were within 15−30 pmol/cm^2 of fixed amino acid labels. From XPS, we determined that 0.5−1% of the polymer units were derivatized in about 50 Å depth. This study establishes practical conditions for the covalent anchorage of biologically active molecules on PET samples.

Introduction

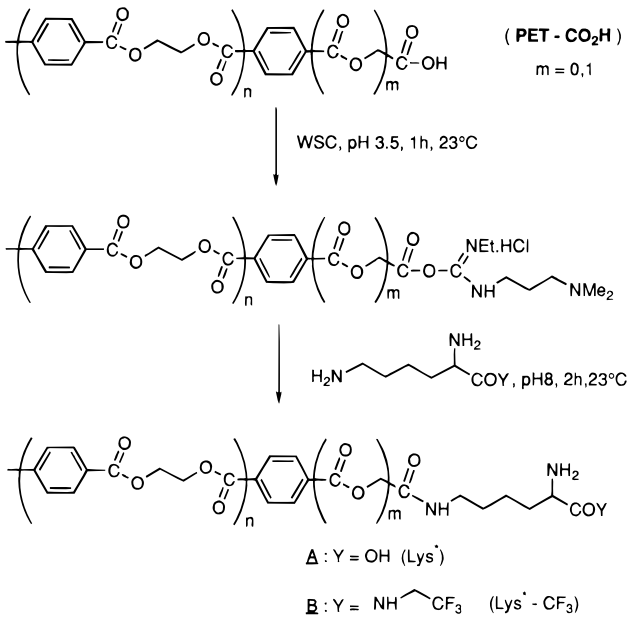
Poly(ethylene terephthalate) (PET) is a synthetic aromatic polyester widely used as a biomaterial[1] for making nonresorbable sutures, or tendon, ligament, and facial implants. Actually, the most successful medical application of PET belongs to reconstructive arterial surgery:[2] vascular grafts of large to medium diameter are manufactured from Dacron using a wide variety of processes. Microporous track-etched membranes made from PET are also currently used as substrates for cultivating mammalian cells.[3]

To improve their biomedical and biotechnological applications, the polymer surfaces are often purposely modified by physical or chemical treatments.[4] In particular, the bioadhesive properties which represent an important aspect of polymer biocompatibility[5] can be tailored by covalent grafting of selected proteins or peptides.[6] Several multistep strategies have been proposed in the previous literature for the anchorage of bioactive molecules on the surface of solid PET samples.[7−12] Recently, the covalent bonding of Dacron with an artificial subendothelium has been described;[13] the chemical process involves the hydrazinolysis of PET ester bonds and their conversion to acyl azides, followed by reaction with connective matrix proteins.

We have already demonstrated that the carboxyl chain ends, naturally displayed on the surface of PET film and membranes ($m = 0$), or created by an oxidative treatment ($m = 1$), could be selectively activated by reaction with water soluble carbodiimide (WSC) and further coupled to amino acid derivatives considered as models of bioactive molecules[14] (Scheme 1A). Similarly, the hydroxyl chain ends were selectively activated by treatement with *p*-toluenesulfonyl chloride (ClTs),[15] or 4,4′-methylenebis(phenylisocyanate) (MDPI),[15] and then reacted with amino acids (Scheme 2A).

In this paper, we further examine the possibility of making use of the naturally-occurring hydroxyl chain ends of PET film and membranes to fix biomolecules.

Scheme 1. Activation of PET Carboxyl Chain Ends and Coupling to L-[4,5-^{3}H]Lysine Derivatives



Our purpose is to accurately determine the ratio of functionalization that could be reached in membranes useful as supports for cell cultivation.[3]

Our previously established methodology is based on highly sensitive radiochemical assays:[14,15] the activated PET surfaces were derivatized with ^{3}H-labeled amino acids and the sample-associated radioactivity was measured by liquid scintillation counting (LSC), giving experimental values that could be directly correlated with the ratio of fixed labels (pmol/cm^2) on the open surface, i.e. the apparent (or visible) surface and the internal surface of the pores. Since cultivated cells mainly contact the apparent surface of microporous membranes, we became interested in the selective quantification of the reactive chain ends displayed on the apparent surface.

The utility of X-ray photoelectron spectroscopy (XPS) for analyzing the surface of medical plastics is well

* To whom correspondence should be addressed. Tel: +32-10-472740. Fax: +32-10-474168.

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Scheme 2. Activation of PET Hydroxyl Chain Ends and Coupling to L-[4,5-^{3}H]Lysine Derivatives

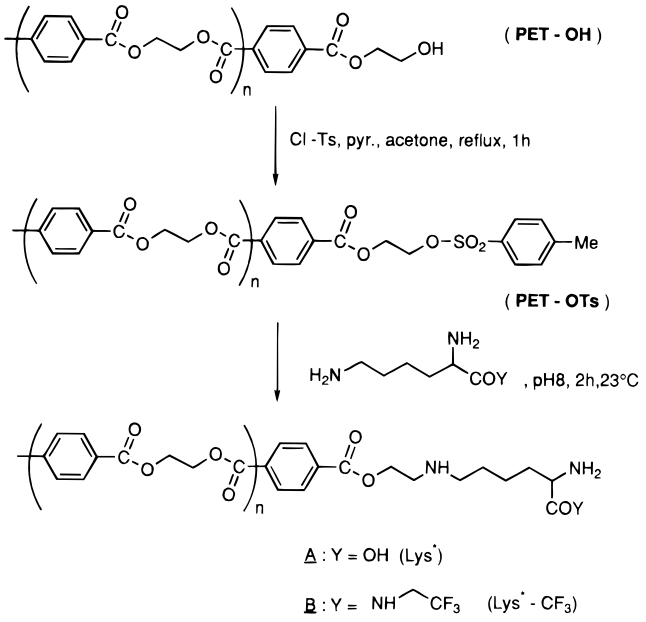
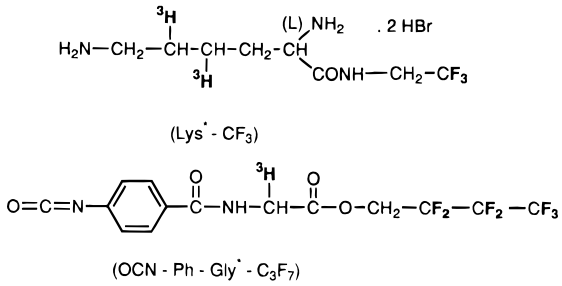


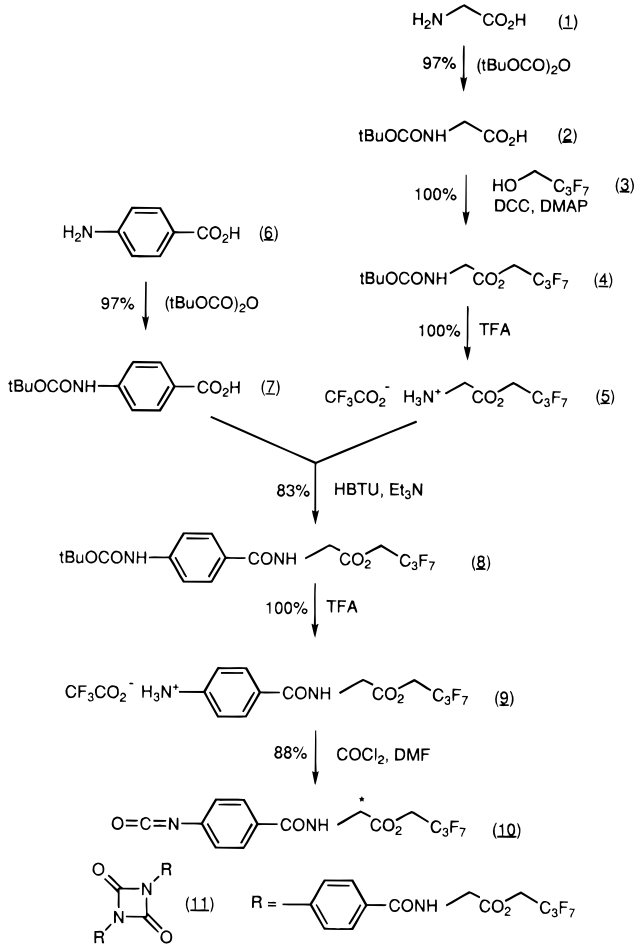
Chart 1. Derivatization Reagents Containing ^{3}H and Fluorine Labels



substantiated.[16] Moreover, the sensitivity of this technique can be significantly enhanced by sample derivatization with reagents containing an elemental tag which is not present in the native polymer; fluorine, with its high photoelectric cross-section, is one of the most popular XPS tags.[17] Thus we designed original derivatization reagents containing ^{3}H and fluorine labels, allowing tandem analysis by LSC and XPS (Chart 1).

L-*N*-(Trifluoroethyl)[4,5-^{3}H]lysinamide (Lys*CF_3) has been already prepared and successfully used for the characterization of PET membranes enriched in carboxyl chain ends (Scheme 1B).[14b] The utility of this dual label is further examplified in this paper with the assay of hydroxyl chain ends (Scheme 2B). In both cases, the PET surfaces have to be adequately activated before coupling to Lys*CF_3. The novel reagent heptafluorobutyl (*p*-isocyanatobenzoyl)[2-^{3}H]glycinate (Chart 1, ONCPhGly*C_3F_7) is now described. This dual label is constructed from *p*-aminobenzoic acid (rigid template), [^{3}H]glycine (LSC label), and perfluorobutanol (XPS tag). Its design is based on the following considerations: (i) the isocyanate moiety should be able to directly react with hydroxyl end groups, without preliminary activation, to give a stable carbamate linkage with the polymer surface; (ii) the F/C ratio determined from XPS analysis of a derivatized sample will represent the reactivity assay of the apparent surface (about 50 Å depth), while the radioactivity counting will give the reactivity assay of the open surface; (iii) the perfluorobutyl ester moiety, displayed on a derivatized surface,

Scheme 3. Synthesis of Heptafluorobutyl (*p*-Isocyanatobenzoyl)[2-^{3}H]glycinate



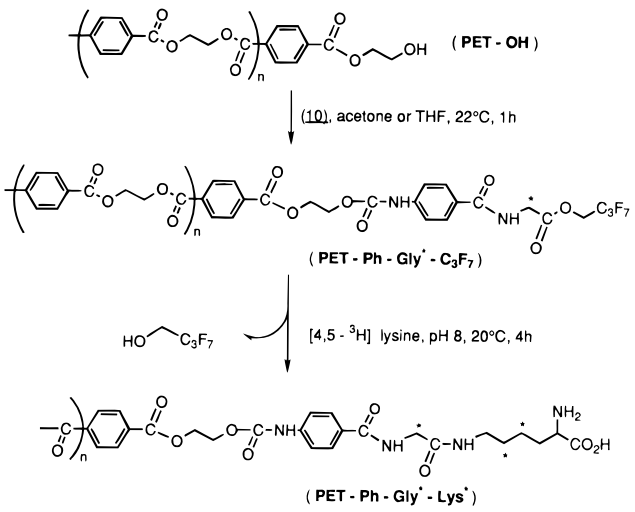
should be sensitive toward nucleophilic substitution with amines (part of biomolecules); (iv) the two reactive functions are separated with a spacer arm (about 12 Å length), probably necessary to reduce steric interations between the surface and the fixed biomolecules. Accordingly, the original analytical tool ONCPhGly*C_3F_7 should also be useful for coupling biologically active molecules on PET membranes.

The chemical characterization of end groups and derivatization of PET samples make use of wet-chemistry procedures, i.e. organic synthesis carried out at the solid−liquid interface.[18] The advantage of this technique is the possibility of performing selective chemical transfomations using a range of mild conditions which restrict the changes to specific surface groups and do not alter the structure of the bulk, nor the pore calibration of track-etched membranes,[19] as confirmed by the scanning electron microscopy (SEM) control.

Results and Discussion

Synthesis of the Derivatization Reagent ONCPhGly*C_3F_7. Glycine **1** containing [^{3}H]glycine (45 ppm) was N-protected by reaction with di-*tert*-butyl dicarbonate,[20] then coupled to 2,2,3,3,4,4,4-heptafluorobutanol (**3**) in the presence of dicyclohexylcarbodiimide and (dimethylamino)pyridine as catalyst.[21] The resulting ester **4** was treated in trifluoroacetic acid to furnish heptafluorobutyl glycinate **5** as the trifluoroacetate salt, in 97% overall yield from glycine **1** (Scheme 3). Several standard methods of peptide synthesis[22]

Scheme 4. Coupling of PET Hydroxyl Chain Ends to Heptafluorobutyl (*p*-Isocyanatobenzoyl)[2-^{3}H]glycinate and Subsequent Ester Displacement with [4,5-^{3}H]Lysine



were investigated for performing the coupling reaction of **5** with *p*-[*N*-(*tert*-butyloxycarbonyl)amino]benzoic acid (**7**);[22,23] the best result was obtained by treatment of a mixture of **7** and **5** in DMF with triethylamine and HBTU.[24] The amide **8** was isolated in 83% yield after two column chromatographies on silica gel. The N-protective group of **8** was cleaved with trifluoroacetic acid, and the resulting anilinium salt **9** was readily converted into isocyanate **10** by reaction with a large excess of phosgene in the presence of dimethylformamide as catalyst.[25] The reaction conducted in hot toluene (90 °C) for 1 h, gave an 88% yield of **10**. Using the conditions of Nowick *et al.*,[26] recently recommended for the synthesis of isocyanates derived from amino acids, we quantitatively recovered the 1,3-diazetidine-2,4-dione dimer **11** ($COCl_2$, pyridine, CH_2Cl_2, 0 °C).

The susceptibility of the heptafluorobutyl ester moiety toward nucleophilic substitution was tested using carbamate **8**: the ester function was stable during 24 h in ethanolic solution and in a mixture of ethyl acetate and water (pH 7−8), but it was rapidly converted into the corresponding amide in the presence of a primary amine (benzylamine in dichloromethane).

LSC Assays of PET Hydroxyl Chain Ends. Three samples were analyzed: native film (F), track-etched microporous membrane with a low density of pores (LD-M), and track-etched microporous membrane with a high density of pores (HD-M). The method A assay (Scheme 2) is a two-step process consisting of the activation of hydroxyl chain ends by tosylation in dry acetone[15] followed by nucleophilic displacement of the resulting tosylates with L-*N*-(trifluoroethyl)[4,5-^{3}H]-lysinamide[14b] in phosphate buffer. The method B assay (Scheme 4) is a one-pot process resulting from the direct reaction of reagent **10** (Scheme 3) in dry acetone with the surface-displayed hydroxyl chain ends. After appropriate washings (see Experimental Section) for removing most of the unreacted ^{3}H reagents, the samples associated radioactivity was measured by liquid scintillation counting (LSC): the results collected in Table 1 are expressed in picomoles per sample and in picomoles per open surface unit (cm^2). In both assays, blank samples were prepared in order to measure the contribution of nonspecific fixation of radioactive labels (irreversible adsorption and diffusion, aminolysis of some PET ester bonds by the NH_2 group of lysine). Subtraction of the blank LSC values from the LSC values recorded for fully treated samples gave the accurate reactivity assays of [OH] end groups, i.e. the number of functions really usable for the covalent anchorage of biomolecules.

The values obtained in method A are approximately half of those recorded in method B. This most probably results from a competition between the hydrolysis of the tosylated endings and their nucleophilic substitution with lysine at pH 8. Thus, the ratios of fixed labels are within 8−15, 30−60, and 80−140 pmol/cm^2 for film, LD membrane, and HD membrane, respectively (Table 1).

In another set of experiments, LD membrane samples were coupled to the reagent **10** in THF solution and the resulting surfaces (PET-PhGly*C_3F_7) were treated with (L)[4,5-^{3}H]lysine in phosphate buffer for 4 h (PET-PhGly*Lys*, Scheme 4). From the sample measured radioactivities (Table 2, entries 2 and 4), we concluded that about 15% of the perfluorobutyl ester groups displayed on the PET-PhGly*C_3F_7 membranes have been substituted with lysine considered as a model of biologically active peptides; thus, the ratio of derivatization that could be reached by this procedure is about 14 pmol/cm^2. According to Massia and Hubbell, a surface concentration of 12 pmol/cm^2 of grafted peptides was large enough to provide a good cell-adhesive substrate from a modified glass.[27]

XPS Analysis of Derivatized PET Samples. PET membranes activated by tosylation and coupled to the derivatization reagent Lys*CF_3 (Scheme 2 and Chart 1) were analyzed by X-ray photoelectron spectroscopy (XPS) under standard conditions (see Experimental Section) in order to determine the interface (±50 Å depth) atomic composition (Table 3). Blank samples were prepared (entries 1, 2, 5, and 6); they showed no contamination with sulfur or fluorine atoms. Thus, these elements could be used to calculate the percentages of modified polymer units. The surface of LD and HD membranes treated with ClTs and pyridine (PET-OTs) well contained sulfur atoms; the experimental S/C atomic ratios were 0.002 42 and 0.001 93, respectively (entries 3 and 7). Considering the native polymer unit $C_{10}H_8O_4$ and the modified chain-end unit $C_{17}H_{15}O_6S$, we calculated that about 2.5% and 2.0% of the interface polymer units have fixed the tosyl group, for the LD-M and HD-M samples, respectively.[28] After incubation in a 10^{-3} M aqueous solution of Lys*CF_3 (pH 8), the PET-OTs samples were partially substituted to give PET-Lys*CF_3; the surface of the derivatized LD and HD membranes showed the incorporation of fluorine atoms. The F/C atomic ratios were 0.002 84 and 0.004 14, respectively (entries 4 and 8). The competitive basic hydrolysis of the activated intermediates PET-OTs appeared clearly since the percentages of derivatization calculated from the F/C atomic ratios were significantly lower than 2.5% and 2.0%; we determined 1.0% and 1.4% of polymer units coupled to the amino acid derivative for LD-M and HD-M samples respectively,[29] considering the native unit $C_{10}H_8O_4$ and the modified unit $C_{18}H_{23}O_4N_3F_3$.

The direct coupling of OCNPhGly*C_3F_7 (reagent **10**) gave PET samples of which the surfaces contained fluorine atoms; the F/C atomic ratios determined from XPS analysis (Table 3) were 0.004 06, 0.008 82, and 0.013 79, respectively, for film, LD-M, and HD-M samples (entries 10, 12, and 14). The corresponding percentages of modified polymer units of structure $C_{24}H_{18}O_8N_2F_7$ are 0.6%, 1.3%, and 2.0%.[30] The blank samples (entries 9,

Table 1. LSC Assays of PET Hydroxyl Chain Ends

		method A 1. ClTs, pyr (acetone, reflux, 1 h) 2. Lys*CF_3 (10^{-3} M, pH 8, 2 h, 23 °C)			method B OCN-PhGly*C_3F_7 (50 mM, acetone, 22 °C, 1 h)		
entry	sample	pmol/sample	pmol/cm^2 open surface	[OH], pmol/cm^2	pmol/sample	pmol/cm^2 open surface	[OH], pmol/cm^2
1	F blank	68.4 (44.7)	25.7 (16.8)		1.6 (0.5)	0.6 (0.2)	
2	**F**	88.8 (6.9)	33.4 (2.6)	**7.7**	41.8 (2.1)	15.7 (0.8)	**15.1**
3	LD-M blank	69.2 (19.9)	23.0 (2.2)		8.4 (2.4)	2.8 (0.8)	
4	**LD-M**	155.3 (25.6)	51.6 (8.5)	**28.6**	210.7 (16.9)	70.0 (5.6)	**67.2**
5	HD-M blank	276.4 (15.7)	12.3 (0.7)		8.9 (2.2)	0.4 (0.1)	
6	**HD-M**	2076 (85)	92.4 (3.8)	**80.1**	3229 (130)	143.7 (5.8)	**143.3**
		blank: PET samples treated with pyridine in refluxing acetone for 1 h, then incubated with Lys*CF_3 (10^{-3} M, pH 8, 2 h, 23 °C)			blank: PET samples incubated with BocNH-Ph-$CONHCH_2COOCH_2$*C_3F_7 (**8**) (50 mM in acetone, 1 h, 22 °C)		

Table 2. LSC Assay of PET-PhGly*Lys*

	sample	pmol/sample	pmol/cm^2 open surface	[Lys*], pmol/cm^2
1	LD-M blank 1[a]	12.6 (2.1)	4.2 (0.7)	
2	LD-M-PhGly*C_3F_7[b]	283.5 (45.6)	94.2 (15.1)	
3	LD-M blank 2[c]	41.8 (8.7)	13.9 (2.9)	
4	LD-M-PhGly*Lys*[d]	356.1 (53.3)	118.3 (17.7)	**14.4**

[a] Membrane incubated with **8***, 50 mM in THF, 1 h, 22 °C. [b] Membrane incubated with the isocyanate **10***, 50 mM in THF, 1 h, 22 °C. [c] LD-M blank 1 incubated with Lys*, 10^{-3} M, pH = 8, 4 h, 22 °C. [d] LD-M-PhGly*C_3F_7 incubated with Lys*, 10^{-3} M, pH = 8, 4 h, 22 °C.

11, and 13) were obtained by incubation of the PET disks in a solution of $BocNHPhCONHCH_2CO_2CH_2C_3F_7$ (**8**) in acetone; this precursor of **10** (Scheme 3) still contains the fluorine tag, but not the reactive isocyanate moiety. Thus, some adsorption might be observed, but not covalent coupling with the hydroxyl chain ends. From XPS analysis, we did not detect the presence of fluorine atoms on these blank surfaces, indicating that the physical interactions can be neglected with respect to the chemical reaction of the label with the polymer surface.

As previously, the HD-M samples appeared more derivatized than the LD-M and film samples. Moreover, the F/C values recorded in the series of experiments applying the method B assay (Scheme 4) are higher than the corresponding values obtained according to the method A assay (Scheme 2); indeed, the samples are not exposed to hydrolysis conditions in method B.

Finally, we examined by XPS the LD-M sample successively derivatized with OCNPhGly*C_3F_7 (**10**) and incubated into a 10^{-3} M aqueous solution of [^{3}H]lysine at pH 8 (Table 3, entry 15). As expected, most of the fluorine atoms were removed from the surface; this results from heptafluorobutyl ester substitution and hydrolysis.[31] After 4 h of incubation, 62% of the ester functions of the LD-M-PhGly*C_3F_7 sample have been transformed.[32] From the LSC analysis, we have previously established that hydrolysis was the main reaction, and nucleophilic substitution with lysine, the minor one.

SEM Analysis. We have examined by scanning electron microscopy (SEM) the morphology of the calibrated pores in chemically modified LD membranes (cell culture supports). After fixation of the derivatization reagent OCNPhGly*C_3F_7 (**10**) (Figure 1) and the subsequent treatment with [^{3}H]lysine (Figure 2), the pores remained very regular and well calibrated. Thus, the mild conditions used in the wet-chemistry procedures are particularly suitable for the controlled surface modification of track-etched membranes.

Conclusion

We have validated original derivatization reagents useful for surface reactivity assays of PET film and membranes. Our labels are dual tools, including ^{3}H and fluorine tags, allowing tandem analysis by LSC and XPS.

Track-etched membranes naturally displayed a higher number of reactive hydroxyl chain ends as compared to the native film. The LSC experimental values recorded per samples have been referred to their respective open surfaces; this allows a rigorous comparison between the different samples and between the analytical techniques. LD and HD membranes appeared about 4 times and 10 times as functionalized as the film reference, respectively (Table 1).

From the XPS analyses, we calculated that about 0.6%, 1.3%, and 2% of the polymer units could be modified (maxima values), in film and LD and HD membranes, respectively (Table 3). With this analytical technique, the membranes appeared about 2 times and 3 times as functionalized as the film reference, for LD and HD samples, respectively. Under usual conditions, the depth of XPS investigation is around 50 Å, i.e. about ten atomic layers. Assuming from crystallographic data[33] that one PET repeated unit should occupy a volume of 2.91×10^{-22} cm^3, we calculated the average of 1.72×10^{15} PET units contained in the interface volume "covered" by 1 cm^2 of apparent surface and accessible to the XPS analysis. This value corresponds to 2850 pmoles of [$C_8H_{10}O_4$] per cm^2.[14,15] Accordingly, 1%−2% of interface (50 Å depth) modification would give, in the LSC assay, values of 28−56 pmol/cm^2 of fixed label. Since the LSC measurements, using the label OCNPhGly*C_3F_7 (**10**), are 2−2.5 times as high as the calculated values,[34] we assume that the depth of the chemically modified interface is within 100−125 Å. The lipophilic character of the derivatization reagent **10** could explain this result. The same model can be proposed for the film and LD and HD membrane samples; indeed, the "empty volumes" (not explored by XPS) resulting from the presence of regular micropores in the membranes can be neglected with respect to the analyzed "filled volumes".[35]

The ratios of derivatization which can be reached, using the PET hydroxyl chain ends as anchorage points, are high enough for developing biocompatibilization strategies of LD membranes based on the coupling of active molecules.[36] Method A (Table 1) offers about 30 pmol/cm^2 of fixed labels, while method B followed by nucleophilic substitution (Table 2) offers about 15 pmol/cm^2 of fixed labels via a short spacer arm.

The analytical methodologies we propose in this paper could certainly be useful for the chemical characterization of other polymeric surfaces displaying carboxyl, hydroxyl, or amine functions. The precise nature of the physico-chemical properties which govern the biocompatibility of synthetic polymers, in given biological

Table 3. XPS Analysis of Derivatized PET Samples

entry	sample	elements (%)					atomic ratio		S or F derivatized polymer units (%)
		C_{1s}	O_{1s}	S_{2p}	N_{1s}	F_{1s}	S/C	F/C	
1	LD-M blank 1[a]	74.73	24.24		0.94				
2	LD-M blank 2[b]	74.47	25.01		0.45				
3	LD-M-OTs	74.37	24.23	0.18	1.17		0.002 42		2.5
4	**LD-M-LysCF$_3$**	73.93	24.75	0.15	0.91	0.21		0.002 84	**1.0**
5	HD-M blank 1[a]	74.89	24.21		0.64				
6	HD-M blank 2[b]	75.21	24.39		0.37				
7	HD-M-OTs	72.49	26.55	0.14	0.60		0.001 93		2.0
8	**HD-M-LysCF$_3$**	74.70	23.99	0.04	0.70	0.31		0.004 14	**1.4**
9	F blank 3[c]	73.60	26.40						
10	**F-Ph-GlyC$_3$F$_7$**	73.77	25.79		0.14	0.30		0.004 06	0.6
11	LD-M blank 3[c]	72.01	27.40		0.59				
12	**LD-M-PhGlyC$_3$F$_7$**	73.70	25.21		0.44	0.65		0.008 82	**1.3**
13	HD-M blank 3[c]	74.61	25.39						
14	**HD-M-PhGlyC$_3$F$_7$**	73.94	24.40		0.65	1.02		0.013 79	**2.0**
15	LD-M-PhGlyLys	74.35	24.88		0.50	0.27		0.003 63	0.5

[a] Blank 1: membrane treated for 1 h in refluxing acetone containing 5% pyridine. [b] Blank 2: blank 1 incubated with LysCF$_3$ (10^{-3} M, pH = 8, 2 h, 23 °C). [c] Blank 3: film or membrane incubated with BocNHPhCONHCH$_2$COOCH$_2$C$_3$F$_7$ (8) (50 mM, acetone, 1 h, 22 °C).

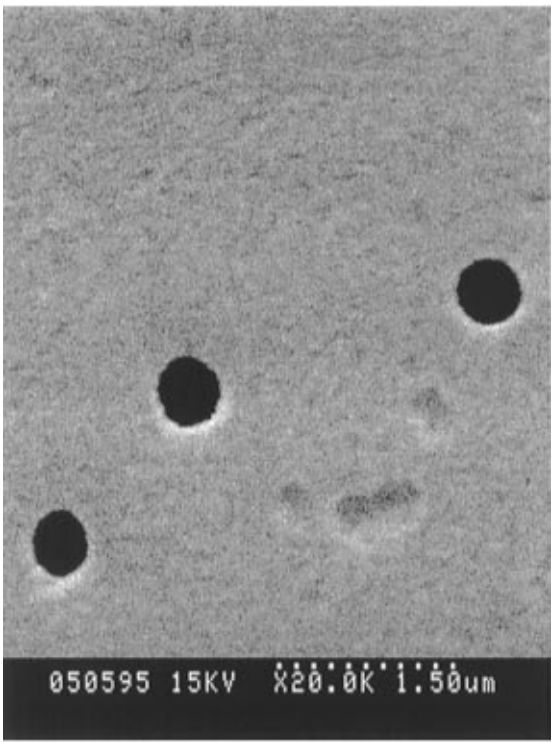


Figure 1. SEM image of the LD membrane derivatized with reagent **10**.

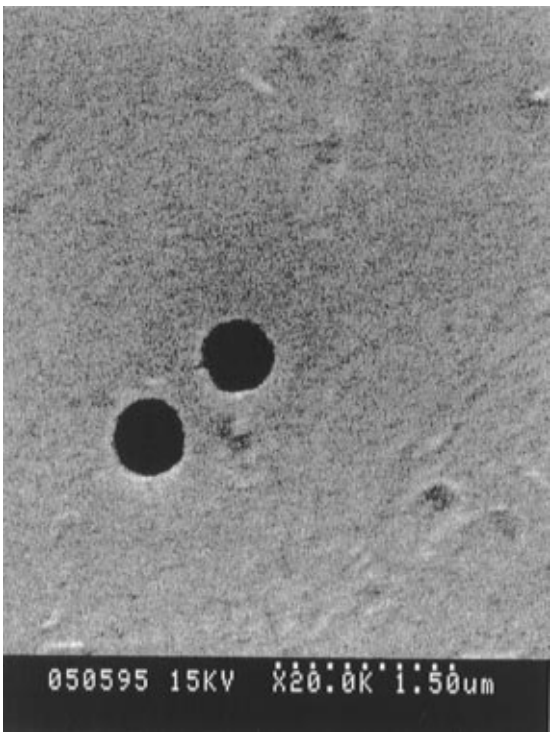


Figure 2. SEM image of the LD membrane derivatized with reagent **10** and subsequently treated with lysine.

environments, remains largely unknown. Therefore, the ability to specifically analyze the surface domain is particularly important.

Experimental Section

Materials and Measurements. The reagents were of analytical grade and purchased from Aldrich (Bornem, Belgium). Triethylamine and pyridine were distilled over CaH_2. *p*-Aminobenzoic acid was recrystallized from water. The radiolabeled amino acids in aqueous solution were purchased from Amersham (Little Chalfont, U.K.); the specific activities were 19.2 and 82 Ci/mmol for [2-^{3}H]glycine and L-[4,5-^{3}H]lysine monohydrochloride, respectively.

CH_2Cl_2 was 99.6% pure and used as such. Diethyl ether, THF, and toluene were distilled over Na. DMF was distilled over CaH_2. Acetone was distilled over $CaSO_4$. Ethyl acetate was 99.8% pure and used as such. Water (HPLC grade) was obtained with a Milli-GQ system (Millipore, Bedford, MA). Citrate buffer (0.1 M, pH 4.2) was prepared from citric acid monohydrate (10.507 g, 50 mmol) and NaOH (2.76 g, 69 mmol) in water (500 mL). Phosphate buffer (0.1 M, pH 8) was prepared from Na_2HPO_4 (8.43 g, 47.35 mmol) and NaH_2PO_4 (0.756 g, 2.65 mmol) in water (500 mL).

The PET film was Mylar A from du Pont de Nemours (Brussels, Belgium) characterized by a thickness of 12 μm and a density of 1.39 g/cm^3. The PET microporous membranes, provided by Whatman SA (Louvain-la-Neuve, Belgium), consisted of the previous film tracked by heavy ions accelerated in a cyclotron and then treated with chemicals (etching by successive immersion in solutions of hydrogen peroxide, sodium hydroxide, and acetic acid and then in water) to obtain calibrated pores.[19] The membrane with a low density of pores (LD-M) contained 1.45×10^6 pores/cm^2 (apparent surface), with a mean diameter of 0.49 μm. The membrane with a high density of pores (HD-M) contained 9.8×10^7 pores/cm^2 (apparent surface), with a mean diameter of 0.41 μm. For surface modifications, PET sample disks of 13 mm in diameter were cut from Mylar A film or from track-etched microporous membranes; the sample open surfaces were 2.66, 3.01, and 22.47 cm^2 for the film disk, the LD-M disk, and the HD-M disk, respectively. The PET samples were used as received, without pretreatment.

Merck silica gel 60 (70−230 mesh ASTM) was used for column chromatography. Melting points (Digital melting point

apparatus, Electrothermal, England) are uncorrected. The IR spectra were taken with a Perkin-Elmer 600 instrument and calibrated with polystyrene (1601 cm^{-1}). The NMR spectra were recorded on Varian Gemini 200 and 300 spectrometers with tetramethylsilane as the internal standard. The mass spectra were obtained with a Finnigan MAT TSQ-70 instrument in the fast atom bombardment (FAB) mode (Xe, Ion Tech 8 kV). The microanalyses were performed at the University College of London (Dr. Alan Stones).

The PET sample-associated radioactivity was measured by liquid scintillation counting (LSC). The modified disks were individually placed in 5 mL polystyrene vials (Canberra Packard), and 2 mL of Aqualuma cocktail (Lumac, Basel, Switzerland) was added to each vial. A TriCarb 1600 TR liquid scintillation analyzer (Packard Instruments, San Diego, CA) was used. The experimental values in cpm were converted to dpm using the relationship dpm = cpm/counting efficiency.[14] The results given in the tables are expressed in picomole per sample and in picomole per open surface unit (cm^2). Each value is the average of, at least, five independent measurements performed with five samples similarly treated. The standard deviation is indicated in brackets.[14]

The surface chemical composition of the PET disks was determined by X-ray photoelectron spectroscopy (XPS) using a SSI X-probe (SSX-100/206) spectrometer from Fisons equipped with an aluminum anode (10 kV, 17 mA) and a quartz monochromator. The direction of photoelectron collection made angles of 55 and 73° with the normal to the sample and the incident X-ray beam, respectively. The electron flood gun was set at 6 eV. Detailed scans of O_{1s}, C_{1s}, N_{1s}, S_{2p}, and F_{1s} lines were performed with a 600 or 1000 μm spot and a pass energy of 150 eV. It was followed by a wide scan (0−1000 eV binding energy) performed in the same conditions. The number of repeated scans were five for C_{1s} (284.8, 286.3, 288.7, and 291.5 eV), five for O_{1s} (531.6 and 533.2 eV), 10 for N_{1s} (399.8 eV), 10 for S_{2p} (169.8 eV ($S_{2p}^{1/2}$) and 168.6 eV ($S_{2p}^{3/2}$), in the ratio 1:2), and 32 for F_{1s} (688.4 eV). During the analyses, the vacuum in the chamber was between 2.5×10^{-6} and 2.5×10^{-7} Pa. The conditions selected for the analysis allowed us to achieve a resolution of 1.5 eV full width at half-maximum (FWHM) on the $Au_{4f7/2}$ line for gold standard. The work function of the spectrometer was determined with the $Au_{4f7/2}$ peak at a binding energy of 83.98 eV. The binding energy of the analyzed peaks was determined by setting the C_{1s} component due to carbon only bound to carbon and hydrogen at a value of 284.8 eV. The peak area was determined with a nonlinear background subtraction. Intensity ratios were converted into atomic concentration ratios by using the SSI ESCA 8.3D software package.[14,15]

Synthesis of the Derivatization Reagent 10. *N*-(*tert*-Butyloxycarbonyl)[2-^{3}H]glycine, 2. Into a 50 mL round-bottomed flask equipped with a magnetic stirrer were successively introduced [2-^{3}H]glycine (**1***) (aqueous solution, 6 mL, radioactive concentration 1.0 mCi/mL, 0.312 μmol), glycine (**1**) (532 mg, 6.94 mmol), NaOH (305 mg, 7.63 mmol, 1.1 equiv) in water (7.5 mL), and *tert*-butanol (10.5 mL). The temperature of the mixture rose to 30 °C. Di-*tert*-butyl dicarbonate (1.562 g, 6.94 mmol, 1 equiv) was added under vigorous stirring, during 10 min. The mixture was left overnight at room temperature, then cooled to 0 °C, and acidified to pH 2.5 with aqueous $KHSO_4$. The mixture was extracted with diethyl ether (4 × 10 mL). The organic phase was diluted with CH_2Cl_2, dried over $MgSO_4$, and concentrated under vacuum to give a white solid **2**. Yield: 1.204 g (97%). Mp: 88−91 °C.

IR (KBr): 3410, 3343, 3115, 2981, 1746, 1671, 1537, 1454, 1413, 1370, 1337, 1282, 1255, 1199, 1166, 1058, 959, 859 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (s, 9H, tBu), 3.96 and 3.91 (d, 2H, CH_2), 6.81 and 5.10 (br s, 1H, NH), 9.91 (br s, 1H, CO_2H) (two conformers). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.1, 42.0 and 43.2, 80.3 and 81.7, 156.1 and 157.3, 173.9 and 174.3 (two conformers).

2,2,3,3,4,4,4-Heptafluorobutyl *N*-(*tert*-Butyloxycarbonyl)[2-^{3}H]glycinate, 4. Into a 50 mL round-bottomed flask equipped with a magnetic stirrer and an addition funnel were successively introduced BocGly* (**2**) (1.184 g, 6.76 mmol), CH_2Cl_2 (7 mL), heptafluorobutanol (**3**) (0.845 mL, 6.76 mmol) and (dimethylamino)pyridine (83 mg, 0.676 mmol, 0.1 equiv). The mixture was cooled to 0 °C, and dicyclohexylcarbodiimide (1.673 g, 8.11 mmol, 1.2 equiv) in CH_2Cl_2 (7 mL) was added dropwise during 1 h 30 min, under stirring. The mixture was further stirred for 30 min at 0 °C and 30 min at room temperature. The dicyclohexylurea was filtered off and washed with CH_2Cl_2 (20 mL). The filtrate was concentrated and treated at 0 °C with ethyl acetate to crystallize the remaining urea. Filtration and concentration gave the ester **4** which was used in the next step without purification. Yield: 2.69 g (100%), colorless oil.

IR (film): 3352, 2982, 2938, 1778, 1704, 1650, 1519, 1456, 1429, 1396, 1370, 1355, 1287, 1231, 1208, 1158, 1131, 1106, 1058, 1022, 949, 912, 826, 739 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.5 (s, 9H, tBu), 4.0 (d, J = 5.9 Hz, 2H, CH_2N), 4.6 (tt, J_{H-F} = 13.4 Hz and 2.6 Hz, 2H, OCH_2), 5.0 (br s, 1H, NH). ^{13}C NMR (75 MHz $CDCl_3$): δ 28.1, 42.0, 59.7 (t, $^2J_{C-F}$ = 27 Hz), 80.2, 108.5 (Tqt, $^1J_{C-F}$ = 264 Hz, $^2J_{C-F}$ = 38.5 Hz and 34.8 Hz), 113.7 (Tt, $^1J_{C-F}$ = 255 Hz, $^2J_{C-F}$ = 31 Hz), 117.4 (Qt, $^1J_{C-F}$ = 285 Hz, $^2J_{C-F}$ = 33 Hz), 155.7, 169.1. MS (FAB), *m*/*e* (%): 358 (M + 1, 31), 302 (100), 258 (43). Anal. Calcd for $C_{11}H_{14}NO_4F_7$: C, 36.99; H, 3.95; N, 3.92. Found: C, 37.01; H, 3.99; N, 3.89.

2,2,3,3,4,4,4-Heptafluorobutyl Glycinate 5. The crude ester **4** (6.76 mmol) was dissolved in trifluoroacetic acid (7 mL), and the solution was stirred for 30 min at room temperature. Concentration and co-evaporation with ethyl acetate (3 × 50 mL) under vacuum gave a pale pink solid **5**. Yield: 2.51 g (100%, trifluoroacetate salt).

^{1}H NMR (300 MHz, DMSO-d_6): δ 4.00 (s, 2H, CH_2N), 5.00 (t, J_{H-F} = 21 Hz, 2H, CH_2O), 8.45 (br s, 3H, NH_3^+). ^{13}C NMR (75 MHz, DMSO-d_6): δ 39.4, 59.4 (t, $^2J_{C-F}$ = 26.2 Hz), 108.1 (Tqt, $^1J_{C-F}$ = 263.2 Hz, $^2J_{C-F}$ = 37.8 Hz and 34.1 Hz), 113.8 (Tt, $^1J_{C-F}$ = 254.5 Hz, $^2J_{C-F}$ = 30.9 Hz), 115.9 (Q, $^1J_{C-F}$ = 289.9 Hz), 117.1 (Qt, $^1J_{C-F}$ = 286.7 Hz, $^2J_{C-F}$ = 33.5 Hz), 159.0 (q, $^2J_{C-F}$ = 35.7 Hz), 166.8. MS (FAB), *m*/*e* (%): 258 ($C_6H_6NO_2F_7$ + 1, 100).

4-[*N*-(*tert*-Butyloxycarbonyl)amino]benzoic Acid 7. Into a 250 mL round-bottomed flask equipped with a magnetic stirrer were successively introduced *p*-aminobenzoic acid (**6**) (5.496 g, 0.04 mol), NaOH (1.76 g, 0.044 mol, 1.1 equiv) in water (44 mL), and *tert*-butanol (60 mL), and di-*tert*-butyl dicarbonate (9 g, 0.04 mol) was added under vigorous stirring during 30 min. The mixture was further stirred for 24 h, then cooled at 0 °C, and acidified to pH 2−3 with aqueous $KHSO_4$. Extraction with diethyl ether (4 × 100 mL), drying over $MgSO_4$, and concentration gave the carbamate **7** which was finally dried by co-evaporation with toluene (2 × 100 mL). Yield: 9.2 g (97%), amorphous beige solid.

IR (neat): 3370, 2982, 1708, 1681, 1610, 1591, 1525, 1508, 1447, 1424, 1411, 1390, 1370, 1312, 1289, 1234, 1161, 1057, 1031, 1019, 944, 907, 859, 840, 801 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.6 (s, 9H, tBu), 7.51 (d, J = 8.7 Hz, 2H), 7.93 (br s, 1H, NH), 7.99 (d, J = 8.7 Hz, 2H).

2,2,3,3,4,4,4-Heptafluorobutyl [(*N*-(*tert*-Butyloxycarbonyl)amino)benzoyl][2-^{3}H]glycinate, 8. To a cold solution (0 °C) of **5** (6.76 mmol, trifluoroacetate salt) in DMF (40 mL) were added the acid **7** (6.414 g, 27.036 mmol, 4 equiv) and *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) (10.253 g, 27.036 mmol, 4 equiv). Under vigorous stirring, triethylamine (4.695 mL, 33.795 mmol, 5 equiv) was added dropwise. The mixture was stirred for 15 min at 0 °C and for 5 h at room temperature. After addition of ethyl acetate (80 mL), the solution was washed with brine (4 × 40 mL), dried over $MgSO_4$, and concentrated under vacuum to give a brown oil which was rapidly filtered through a silica gel column (60 g) with a mixture of CH_2Cl_2/ethyl acetate/petroleum ether (5:1:2) as eluent. Column chromatography on silica gel (300 g) with the previous solvent mixture furnished pure **8**. Yield: 2.67 g (83%, amorphous beige solid). Mp: 172−173 °C (recrystallization from ethyl acetate/hexane, 1:2).

IR (neat): 3352, 2984, 2941, 1779, 1704, 1650, 1614, 1591, 1520, 1464, 1448, 1407, 1369, 1357, 1308, 1231, 1161, 1126, 1059, 1027, 1002, 913, 850, 798, 772, 762, 745 cm^{-1}. ^{1}H NMR: (300 MHz, $CDCl_3$): δ 1.70 (s, 9H, tBu), 4.52 (d, J =

5.15 Hz, 2H, CH_2N), 4.9 (tt, $^3J_{H-F}$ = 13.4 Hz, $^4J_{H-F}$ = 1 Hz, 2H, CH_2O), 6.53 (br t, J = 5.15 Hz, 1H, NH), 6.66 (br s, 1H, $NHCO_2$), 7.45 (d, J = 8.7 Hz, 2H, Ar), 7.76 (d, J = 8.7 Hz, 2H, Ar). ^{13}C NMR (75 MHz, DMSO-d_6): δ 28.1, 41.0, 59.0 (t, $^2J_{C-F}$ = 26.2 Hz), 79.6, 108.0 (Ttq, $^1J_{C-F}$ = 262.8 Hz, $^2J_{C-F}$ = 36.4 Hz), 114.2 (Tt, $^1J_{C-F}$ = 254.3 Hz, $^2J_{C-F}$ = 30.6 Hz), 116.7 (Qt, $^1J_{C-F}$ = 293.8 Hz, $^2J_{C-F}$ = 30.5 Hz), 117.18, 126.71, 128.23, 142.75, 152.64, 166.33, 169.00. MS (FAB), *m/e* (%): 477 (M + 1, 31), 421 (7), 420 (24), 403 (5), 376 (24), 220 (28), 180 (6), 165 (8), 164 (100). Anal. Calcd for $C_{18}H_{19}N_2O_5F_7$: C, 45.39; H, 4.02; O, 5.88. Found: C, 46.16; H, 4.27; O, 5.81.

2,2,3,3,4,4,4-Heptafluorobutyl (*p*-Aminobenzoyl)[2-^{3}H]-glycinate, 9. Compound **8** (768 mg, 1.612 mmol) was dissolved in trifluoroacetic acid (5 mL) and stirred for 30 min at room temperature. Concentration and co-evaporation with ethyl acetate (4 × 40 mL) under vacuum afforded a pink solid **9** which was used as such in the next step. Yield: 790 mg (100%, trifluoroacetate salt).

^{1}H NMR (200 MHz, DMSO-d_6): δ 4.7 (s, 2H, CH_2N), 5.0 (m, $^3J_{H-F}$ = 13.2 Hz, 2H, CH_2O), 7.9−7.8 (m, 2H, Ar), 8.3−8.2 (m, 2H, Ar). ^{13}C NMR (75 MHz, DMSO-d_6): δ 41.1, 59.0 (t, $^2J_{C-F}$ = 26 Hz), 108.31 (Ttq, $^1J_{C-F}$ = 264.0 Hz, $^2J_{C-F}$ = 30.1 Hz and 38.0 Hz), 114.3 (Tt, $^1J_{C-F}$ = 254.0 Hz, $^2J_{C-F}$ = 30.4 Hz), 115.5 (Q, $^1J_{C-F}$ = 288.2 Hz), 117.3 (Qt, $^1J_{C-F}$ = 285.9 Hz, $^2J_{C-F}$ = 33.8 Hz), 116.3, 124.7, 129.1, 146.5, 158.64 (q, $^2J_{C-F}$ = 37.2 Hz), 166.55, 169.15. MS (FAB), *m/e* (%): 377 ($C_{13}H_{11}N_2O_3F_7$ + 1, 23), 376 (26), 136 (33), 120 (100).

2,2,3,3,4,4,4-Heptafluorobutyl(*p*-Isocyanatobenzoyl-[2-^{3}H]glycinate, 10. A mixture of **9** (1.612 mmol, trifluoroacetate salt) and phosgene in toluene (1.93 M, 38.4 mL, 74.11 mmol, 46 equiv) was heated at 90 °C for 1 h. Evaporation left an oil which was partly dissolved in CH_2Cl_2. Filtration and concentration gave the isocyanate **10**. Yield: 587 mg (88%, pale yellow solid).

IR (CH_2Cl_2): 3380, 2366, 1771, 1672, 1650, 1608, 1510 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.36 (d, J = 5.4 Hz, 2H, CH_2N), 4.70 (t, $^3J_{H-F}$ = 13.9 Hz, 2H, CH_2O), 6.80 (br s, 1H, NH), 7.45 (d, J = 8.5 Hz, 2H, Ar), 7.79 (d, J = 8.5 Hz, 2H, Ar).

Specific radioactivity: 6.1×10^{11} dpm/mol or 1.5×10^9 dpm/g.

Procedures for Surface Chemistry. PET-OH Tosylation and Coupling to L-*N*-(Trifluoroethyl)[4,5-^{3}H]lysinamide (Method A). The glassware was cleaned with sulfochromic acid before use and dried at 160 °C for 15 h. The activation reaction was performed under an argon atmosphere in a two-necked 100 mL flask equipped with a reflux condenser and a magnetic stirrer (5 mm × 30 mm). Five PET disks were treated by immersion into a solution of tosyl chloride (1 g, 5.2 mmol) and pyridine (0.424 mL, 5.2 mmol) in dry acetone (20 mL). The samples were stirred under reflux during 1 h, then taken off the reactive solution with tweezers, and drained over filter paper. The disks were successively washed, under shaking (Edmund Bülher stirrer, model KL-2), with acetone (50 mL, 10 min), water (50 mL, 10 min), pH 4.2 citrate buffer (50 mL, 5 min, two times), and water (50 mL, 5 min). The disks (PET-OTs) were drained over filter paper and directly assayed with L-*N*-(trifluoroethyl)[4,5-^{3}H]lysinamide (Lys*CF_3). The reagent has been prepared according to Deldime *et al.*[14b] as dihydrobromide salt (specific radioactivity: 1.09×10^{13} dpm/mol or 2.79×10^{10} dpm/g); a 10^{-3} M solution of Lys*CF_3 in phosphate buffer (pH 8) was used for the PET samples labeling. The disks were individually treated in small tubes (16 mm × 100 mm) containing 1.5 mL of the previous solution, for 2 h at 23 °C, under shaking. The lysine solution was removed by sucking and replaced by the washing solutions. Each disk was successively washed, under shaking, with 1.5 mL of pH 8 phosphate buffer (2 × 5 min), water (5 min), 0.005 N HCl (10 min, then 2 × 5 min), and water (3 × 5 min). The disks were drained over filter paper and directly analyzed by liquid scintillation counting (PET-Lys*CF_3).

Blank Samples: The reference samples, for the counting of the nonspecific fixation of Lys*CF_3, were prepared according the previous procedure, but in the activation step, the tosyl chloride reagent was omitted.

Weight Control: Our wet-chemistry procedure allowed the designed surface derivatization without extensive erosion of the PET samples; the weight loss was lower than 4%.

PET-OH Coupling to Heptafluorobutyl (*p*-Isocyanatobenzoyl)[2-^{3}H]glycinate (Method B). The acylation reaction was performed under an argon atmosphere in a two-necked 100 mL flask equipped with a magnetic stirrer (6 mm × 30 mm). Five PET disks were treated by immersion into a solution of heptafluorobutyl (*p*-isocyanatobenzoyl)[2-^{3}H]glycinate (**10**) (412 mg, 1 mmol) in acetone or THF (25 mL). The samples were stirred for 1 h at room temperature, then taken off the reactive solution with tweezers, and drained over filter paper. The disks were washed, under shaking (Edmund Bülher stirrer, model KL-2), with acetone or THF (50 mL, 10 min and 4 × 5 min), then drained over filter paper and directly analyzed by LSC (PET-PhGly*C_3F_7).

Blank Samples. The reference samples, for the counting of the nonspecific fixation of the [^{3}H]glycinate reagent, were obtained by immersion of the disks into a 50 mM solution of **8*** (precursor of **10*** with masked isocyanate function) in acetone or THF, for 1 h at 22 °C, and standard washings.

Weight Control. A weight gain of about 2% was measured.

Coupling of [4,5-^{3}H]Lysine to PET Samples Acylated with 10*. The disks, activated according to method B, were immersed into a solution of lysine (9.2 mg of unlabeled lysine·HCl) in phosphate buffer (pH 8, 50 mL) containing the label (7.5 μL of [4,5-^{3}H]lysine solution/mL). The samples were individually treated in small tubes (16 mm × 100 mm) with 1.5 mL of the previous solution (10^{-3} M Lys*), for 4 h, at 22 °C, under shaking. The lysine solution was removed by sucking and replaced by the washing solutions. Each disk was successively washed, under shaking, with 1.5 mL of pH 8 phosphate buffer (6 × 5 min), water (1 × 5 min), 0.005 N HCl (3 × 5 min), and water (3 × 5 min). The disks were drained over filter paper and directly analyzed by LSC (PET-Ph-Gly*Lys*).

Blank Samples. The blank samples obtained from method B were immersed in the solution of [^{3}H]lysine and washed as described above.

SEM Analysis. Scanning electron microscopy was performed using an Hitachi S-570 system with an accelerating voltage of 15 kV and a working distance of approximately 5 mm. The samples were gold-coated in a Balzers Union SCD 040 vapor deposition unit, at 15 mA for a period of 90 s.

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(29) (a) F/C calcd = 3 × 0.01/(0.99 × 10 + 0.01 × 18) = 0.0029 (F/C experim = 0.0028). (b) F/C calcd = 3 × 0.014/(0.986 × 10 + 0.014 × 18) = 0.0042 (F/C experim = 0.0041).
(30) (a) F/C calcd = 7 × 0.006/(0.994 × 10 + 0.006 × 24) = 0.00416 (F/C experim = 0.00406). (b) F/C calcd = 7 × 0.013/(0.987 × 10 + 0.013 × 24) = 0.00893 (F/C experim = 0.00882). (c) F/C calcd = 7 × 0.02/(0.98 × 10 + 0.02 × 24) = 0.01362 (F/C experim = 0.01379).
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(32) F/C calcd = 7 × 0.005/(0.995 × 10 + 0.005 × 24) = 0.00347. The starting sample contained 1.3% of fluorine tag (entry 12); the final sample contained 0.5% of fluorine tag (entry 15), corresponding thus to 62% of ester transformation with loss of heptafluorobutyl alcohol.
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(34) (a) LD-M-PhGly*C_3F_7: 1.3% of derivatized units from XPS; calcd LSC concentration = 28 × 1.3 pmol/cm^2 = 36.4 pmol/cm^2; experim LSC concentration = 67.2 pmol/cm^2. (b) HD-M-PhGly*C_3F_7: 2% of derivatized units from XPS; calcd LSC concentration = 28 × 2 pmol/cm^2 = 56 pmol/cm^2; experim LSC concentration = 143.3 pmol/cm^2 = 2.56 × 56 pmol/cm^2.
(35) (a) Empty volume for LD-M in 50 Å depth covered by 1 cm^2 of apparent surface: $v = \pi r^2 hn$; $v = 3.1416 \times (0.245 \times 10^{-4})^2 \times (50 \times 10^{-8}) \times (1.45 \times 10^6)$ cm^3 = 13.66×10^{-10} cm^3; that is to say 0.3% of the XPS analyzed volume. (b) Empty volume for HD-M in 50 Å depth covered by 1 cm^2 of apparent surface: $v = 3.1416 \times (0.245 \times 10^{-4})^2 \times (50 \times 10^{-8}) \times (9.8 \times 10^7)$ cm^3 = 6.46×10^{-8} cm^3; that is to say 13% of the XPS analyzed volume.
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