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SYNTHESIS OF DIPYRRINONE ESTERS USING CARBODIIMIDE REAGENTS

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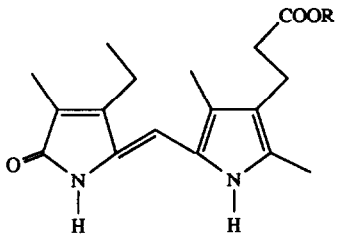
Abstract. *The esters of xanthobilirubic acid (XBR) with rac-(±)-, (R)-(+)-, and (S)-(-)-alpha-methylbenzyl alcohol (1, 2 and 3 respectively), with rac-(±)-, (R)-(+)-, and (S)-(-)-sec-butyl alcohol (4, 5 and 6 respectively), with rac-(±)-, (R)-(+)-, and (S)-(-)-2-octanol (7, 8 and 9 respectively) have been synthesized in good to excellent yields using as condensing agent the mixture dicyclohexylcarbodiimide/ dimethylaminopyridine w(DCCI/ DMAP). These esters are models for the study of exciton coupling in hydrogen-bonded dimers. The esters of XBR with monomethoxypolyethyleneglycol (MPEG) of FW= 1900, and with polyethyleneglycol (PEG) of FW= 2000 (10 and 11 respectively) have been quantitatively prepared by the same procedure. These water-soluble dipyrrinone esters are models for the study of Z ⇌ E photoisomerization in rats. Attempts to extrapolate the procedure to the synthesis of the same esters of bilirubin (BR) have always led to difficult-to-separate mixtures of products.*

INTRODUCTION

Bilirubin (BR) esters are important both biologically, as BR must be glucuronated (i.e., esterified with glucuronic acid) before excretion[1], and chemically, because of the glucuronide's increased water solubility, and because of the esters unusual structural and spectroscopic properties.

Until recently, BR methyl esters were the only commonly used esters in these studies[2]. They can be obtained in good yields by brief treatment of the pigment at room temperature with diazomethane[3]. Alternative procedures requiring strong acids cannot be used because BR undergoes constitutional isomerization, oxidation, and decomposition under these conditions[3]. Other esterification procedures from the literature (i.e., the use of the 1-alkyl-3-p-tolyltriazine reagent[4]) are of much less general application.



$CH_3O-(CH_2CH_2)_n CH_2CH_2OH$

n≈ 41

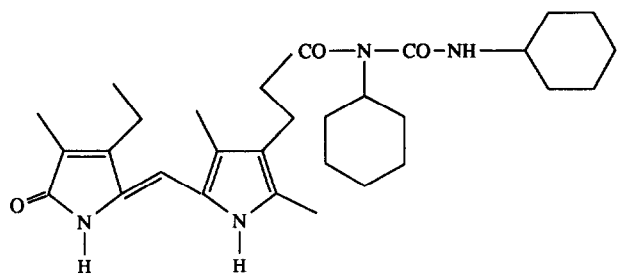
MPEG-1900

$HO(CH_2CH_2)_n-CH_2CH_2OH$

n≈ 44

PEG-2000

Compound	R
1	(rac)-(±)- $CH(CH_3)Ph$
2	(R) - (+)- $CH(CH_3)Ph$
3	(S) - (-) - $CH(CH_3)Ph$
4	(rac)-(±) -$CH(CH_3)CH_2CH_3$
5	(R) - (+)- $CH(CH_3)CH_2CH_3$
6	(S) - (-) - $CH(CH_3)CH_2CH_3$
7	(rac)-(±)- $CH(CH_3)(CH_2)_5CH_3$
8	(R) - (+)- $CH(CH_3)(CH_2)_5CH_3$
9	(S) - (-) - $CH(CH_3)(CH_2)_5CH_3$
10	MPEG-1900
11	PEG-2000)-$_{1/2}$
12	H



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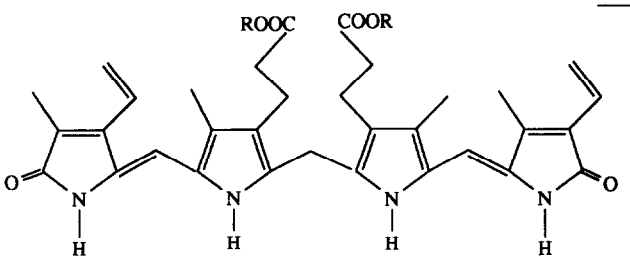
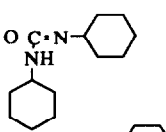
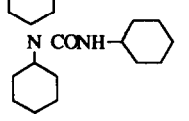


Compound	OR
14	OH
15	O-MPEG-1900
16	
17	

Formula Scheme

On the other hand, our group has successfully exploited the use of Shioiri's reagent diphenylphosphoryl azide[5a] for the preparation of **XBR** amide, N-methylamide and dimethylamide[6]; of chiral **XBR** amides counterparts of the present chiral esters[7], and of bilirubin mono- and bis-dimethylamides[8].

In the present paper, the utility and limitations of the DCCI-mediated synthesis of esters applied to a model of **BR**, the dipyrrinone **XBR** (12) are discussed. The alcohols chosen are such that the resulting esters are of interest in the study of exciton coupling in hydrogen-bonded dimers (as in esters 1 to 9)[9], or as water-soluble models for the investigation of $\underset{\sim}{Z} \rightleftharpoons \underset{\sim}{E}$ photoisomerization in rats (as in compounds 10 and 11)[1].

RESULTS

DCCI or mixtures of DCCI/ DMAP as reagents for the synthesis of esters and amides (i.e., in the synthesis of peptides[10]) have been widely used. However, to our knowledge, no application of the procedure to the synthesis of esters (or amides) of linear tetra- and dipyrroles has been reported.

The standard procedure had to be adapted to the specific behaviour of our pigment carboxylic acids (i.e., low solubility in the solvents most frequently used, which prevents carrying out the reaction at 0°C, and light sensitivity among others). Basically, the optimized procedure for preparing esters 1- 9 involves treatment of a CH_2Cl_2 suspension of **XBR** and the corresponding alcohol (2.5 to 5 times excess) with DCCI/ DMAP. The use of excess alcohol is due to the need to drive the esterification to completeness. On the other hand, such an excess of alcohol will only be useful when it is volatile to some extent, i.e., it can subsequently be removed under vacuum. The reaction and the purity of the final product can be monitored by reverse-phase HPLC by detecting at 400 nm as described in the experimental part. The structure of the target esters are spectroscopically confirmed by IR (ester band near 1730- 1735 cm^{-1}), ^{1}H-NMR (characteristic -CH(CH_3)- for the secondary alcohol), as well as UV- Vis (for the chiral esters, also CD). The low field part of the ^{1}H-NMR spectra in $CDCl_3$ of these esters shows the lactam NH near 11.25 ppm, and the pyrrole NH near 10.30 ppm. These are very similar to the values for the respective methyl ester[2c] and suggest a dimeric disposition. Preliminary CD results for the chiral esters 2 and 3, confirm a bisignate curve in the CD spectra in chloroform (the $\underset{\sim}{(R)}$- isomer, 2, shows a positive peak near λ = 445 nm, and a larger, negative one near λ = 400 nm; the same qualitative results, with opposite signs are given by the $\underset{\sim}{(S)}$- isomer), while the curves were monosignate (the $\underset{\sim}{(R)}$- isomer shows a positive peak near λ = 410 nm, and the $\underset{\sim}{(S)}$- isomer a negative one at the same wavelength) and of much lower intensity in dimethylsulfoxide, DMSO. This confirms the exciton coupling of hydrogen-bonded dimers in the first solvent, but monomeric species in the second; a behaviour which mimicks that found for the parent amides[7]. Interestingly, the magnitude of the CD increased in chloroform in going from a

$5x\ 10^{-3}$ M to $5x\ 10^{-5}$ M solutions, and then sharply dropped at a $5x\ 10^{-6}$ M concentration. In DMSO, the CD was comparatively weaker, indicating an intrinsically feeble CD due to the monomeric unit, and suggesting that the more intense bands found in chloroform are due to the interaction between the monomeric units in the dimer. More complete CD results on these compounds under different conditions, and the implications of these results will be published separately.

Table I summarizes the best results of the esterifications attempted. The highest yields are obtained with the least hindered sec-butyl alcohol, and the worst with alpha-methylbenzyl alcohol. In all cases 7 to 20% yields of amide 13, which arises from coupling **XBR** to DCCI, are obtained. The structure of this amide has been confirmed spectroscopically. Its formation has been interpreted by Zalipsky et al.[11] in terms of a C → N rearrangement from the initially formed DCCI-activated **XBR** ester. If the reaction between each alcohol and this activated **XBR** ester is somehow restricted, the above (slow) rearrangement becomes competitive, and variable percentages of the undesired stable amide 13 are obtained.

Table I: Optimized Yields for the DCCI/ DMAP Mediated Synthesis of XBR Esters of Several Secondary Alcohols[a].

Ester	*Yield of Ester*[b] *(%)*	*Yield of 13 (%)*	*Yield of Other Products (%)*
1	*70*[c]	*20*	*10*
2	*72*	*18*	*10*
3	*85*	*15*	
4	*93*	*7*	
5	*90*	*10*	
6	*91*	*9*	
7	*85*	*15*	
8	*85*[c]	*15*	
9	*85*[c]	*15*	

[a] *Unless otherwise specified, the experimental conditions are those of the experimental part.* [b] *Yields from HPLC. For the sec-butyl and 2-octyl esters, HPLC shows the reaction to be complete after 12 h.* [c] *16 Hours reaction.*

Attempts to use the DCCI/ DMAP procedure to diesterify bilirubins have been unsuccessful. Under the optimized conditions described above, TLC showed no appreciable change even after 48 h at room temperature under magnetic stirring (ca. 15% of a **BR** monomethyl ester type of product, ca. 20% after 60 h, and ca. 25% after 84 h). HPLC of this product showed it to consist of two close series of three isomers, with relative integration 30% and 70%. The presence of "triads" indicates scrambling and each series of compounds might correspond to **BR** monoester- monoacid and/ or monoester- mono-DCCI-amide. It is worth to note that DMAP partly prevents scrambling.

The use of sonication only accelerated the formation of the same mixture of products (80% after 24 h). Other products formed were the target **BR** diester (less than 5%) and bis-DCCI-amide (ca. 15%). When Shioiri's diethyl phosphorocyanidate, DEPC[5b] was used as condensing agent, no improvement of the results was noticed. Neither was effective for the same purpose the use of DCCI/ HOBT (hydroxybenzotriazole)[5c] .

We were attracted to synthesizing the MPEG-1900 and PEG-2000 esters of **XBR** (**10** and **11** respectively) because these esters are expected to exhibit amphiphilic properties. Both MPEG and PEG are soluble in H_2O and CH_2Cl_2, but can be precipitated from a CH_2Cl_2 solution by addition of a 3- 5 fold excess of cold ether[12], and a similar behaviour could be expected for their **XBR** esters. Diester **11** might also be of interest for studying intramolecular interactions (and more particularly exciton coupling) between the two dipyrrinone units directly bound to the initially difunctionalized PEG.

From the preparative point of view, the synthesis of these esters is similar to that outlined in the previous section. The main differences are. 1) the use here of a non-volatile alcohol, and 2) the difficulties in separating any unreacted (polymeric) alcohol from the target ester, due to the predominant effect of the polyethyleneglycol chain on the polarity/ solubility properties of the esters. As a consequence, an excess of alcohol is undesirable now, and, rather, to ensure complete esterification of all starting alcohol, an excess **XBR** will be used. Actually, the optimum results were obtained when 1.6 meq **XBR** and 1.8 meq DCCI were used per 1 meq MPEG (2.2 mmol **XBR** and 2.6 mmol DCCI per mmol PEG). At the end of the reaction, the unesterified acid is separated by simple filtration from a CH_2Cl_2 solution concentrated in ester (**XBR** is only poorly soluble in CH_2Cl_2), followed by extraction of any remaining traces with aq. base, from a CH_2Cl_2 solution. The purity of the products can be checked by reverse-phase HPLC: in agreement with their higher polarity, the target esters have lower retention times under these conditions (see experimental part) than the original acid. The products have also been identified spectroscopically in the IR, esterification results in the disappearance of the -OH band near 3430 cm^{-1}, and appearance of the NH band near 3340 cm^{-1}; more important, three relatively small bands appear in the carbonyl zone· at 1730 (ester C=O), 1660 (lactam C=O) and 1630 (C=C). The low intensity -especially in the monoester **10**- corresponds to the small contribution of the chromophore to the molecular weight of product (10- 15%). ^{1}H-NMR spectra also reflect this small contribution the spectrum of each ester shows, importantly, a singlet near 3.6 ppm due to the polymer -(-CH_2CH_2-O-)$_n$- unit, while the bands of the chromophore appear very small. In the monoester **10**, a singlet near 3.3 ppm due to the terminal -O-CH_3 in the polymer, integrates 1·1 relative to each of the methyl groups of the bound chromophore, confirming one XBR moiety per polymer chain. In the diester **11**, the 2 1 ratio of XBR per polymer chain is confirmed from the relative integration of the chromophore methyl groups compared to the singlet near 3.6 ppm due to the polymer (see experimental part).

Again, our attempts to use the procedure for a practical synthesis of MPEG bilirubin mono- and/ or diesters have failed. Under the best conditions (1.1 meq **BR**, 1.3 meq DCCI, 0.25 meq DMAP in dry CH_2Cl_2, sonication, room temperature, dark, 10 days!!), complex mixtures containing the expected diester **15** (29%), the respective monoester (48%), plus two more products -none of which corresponds to starting **BR** (by reverse-phase HPLC)- are obtained. The course of the reaction can be checked by reverse-phase HPLC: under these conditions, **BR** is slowly converted into the DCCI-activated ester **16** (the reaction takes only 1 h in DMSO). If reaction of MPEG with **16** to give the desired ester **15** is not fast enough, then **16** slowly rearranges to the amide **17** (λ_{max}= 450 nm in both CH_2Cl_2 and in MeOH), a stable product which will not react with MPEG. Isolation and purification of **BR**-MPEG mono- and diester from the reaction mixture and from each other are difficult, and so the mixture of the two has been identified by IR (ester C=O band near 1730 cm^{-1}), and UV- Vis ($\lambda_{max}^{H_2O}$ = 450, 420 (sh); $\lambda_{max}^{CH_2Cl_2}$ = 440 (sh), 405 nm).

A modification of the previous procedure, in which HOBT[5c] was used instead of DMAP, did not improve the results. Nor were these improved using as condensing agent DEPC[5b].

EXPERIMENTAL

All nmr spectra were run on an IBM NR80/AF or JEOL FX-100 FT spectrometer in either deuteriochloroform (99.9% d_1) or dimethylsulfoxide-d_6 (99.9% d_6), both from Aldrich. All uv-visible absorption spectra were run on a Cary 219 instrument. All circular dichroism spectra (500 to 320 nm) were recorded at 19 - 22 ^{o} within 15 min of solution preparation in a JASCO J-600 spectropolarimeter or on a JASCO J-40 spectropolarimeter equipped with a photoelastic modulator; the solvents used ($CHCl_3$ and DMSO) were HPLC grade; in all $CHCl_3$ measurements, scale = 2.5 x 10^{-5} ΔA x cm^{-1}, while in DMSO (dynamic reserve used), scale = 1.5 x 10^{-5} ΔA x cm^{-1}; time constant = 1 s; slit = 2 nm. Analytical thin layer chromatography (tlc) was carried out on J. T. Baker silica gel 1B-F plates (125 micron layer). High performance liquid chromatographic (hplc) analyses used a detector set at 400 nm and a Beckman -Altex Ultrasphere-IP 5 micron C-18 ODS column (25 x 0.46 cm), with a Beckman ODS precolumn (4.5 x 0.46 cm) and a flow of 1 ml/minute of 0.1 M di-n-octylamine acetate in 5% aqueous methanol as eluent[13]. CH_2Cl_2 was solvent grade, distilled shortly before use from K_2CO_3. All solvents and solutions used were rendered oxygen-free, argon-saturated by bringing to brief reflux under a stream of argon, cooling and storing under argon. Reactions were typically carried out under argon.

DCCI, DMAP and DEPC were from Aldrich, HOBT and **BR** were from Sigma, and used without further purification, **XBR** was prepared according to Lightner's procedure[6]; MPEG-1900 (Aldrich) and PEG-2000 (Fluka) were used after removing any traces of water by addition of anhydrous benzene and azeotropic distillation (no weight loss was noticed). (rac)-sec-phenethyl alcohol was obtained (84% yield) by $LiAlH_4$ (Aldrich) reduction of acetophenone (Matheson, Coleman & Bell) in a modification of the procedure previously

reported[14]; (rac)-sec-butyl alcohol (M, C & B), and (rac)-2-octyl alcohol (Sigma) were used without further purification. (R)- and (S)-sec phenethyl alcohol, (R)- and (S)-sec-butyl alcohol and (R)- and (S)-2-octyl alcohol were from Aldrich and used without further purification after spectroscopically confirming their purity.

General Procedure for the Preparation of XBR Esters 1 - 9. The conditions given here have been optimized after several runs. To a mixture of 0.1 meq (30.2 mg) XBR, 0.02 meq (2.5 mg) DMAP and ca. 0.3 meq of the corresponding alcohol in a small glass sample tube equipped with a magnetic bar, 1.5 ml of dry CH_2Cl_2 are added. The tube is stoppered with a polyethylene cap and the mixture let to stir in the dark at room temperature during 5 minutes. In an identical tube, 0.12 meq (24.7 mg) DCCI are dissolved in 1.5 ml of dry CH_2Cl_2. In order to minimize the amount of undesirable byproduct 13, the DCCI solution is added to the mixture of acid, alcohol and DMAP in 4 fractions, with intervals of 4 hours. 16 - 24 h After the first DCCI addition, the dark-yellow suspension is filtered through a 2 ml, 10 -15 M glass filter, leaving behind a pale-yellow solid consisting mostly of dicyclohexylurea, plus some 13. The precipitate is washed with a total of 0.5 ml acetone, and the organic solution evaporated to dryness. Incomplete elimination of DCCI is monitored by a peak in the ir spectrum near 2120 cm^{-1}. The yellow-greenish (alcohol-wetted) solid is kept for 12-24 h in a vacuum dessicator (1 - 5 mm) containing P_2O_5, and the resulting solid purified by refluxing for 10 min in 2 ml acetone, cooling down the solution and filtering off any undesired 13. Only within the sec-phenethyl alcohol series, an additional, unidentified brown impurity is formed, and this can be eliminated by washing the crude with CH_2Cl_2 hexane (1 + 10 ml). The rest of the purification procedure is common for the three series of alcohols, and includes dissolving in refluxing MeOH (1.5 ml), then precipitating the target ester by cooling down the solution, to yield a yellow solid (purity checked by reverse-phase HPLC). For additional data concerning each of the esters prepared, see the following entries.

XBR-(rac)-sec-Phenethyl Ester, 1. Prepared according to the general procedure, from XBR and (rac)-sec-phenethyl alcohol with 70% yield (M.p.= 149- 151°C).
HPLC (see above for conditions)· R_t= 6.1 min. Under these conditions, XBR has R_t= 5.1 min, the undesired amide 13, R_t= 5.6, and the brown, unidentified impurity, R_t= 4.2 min.
IR (KBr, cm^{-1}): 3350 (NH), 2915, 2860, 1735 (ester C=O), 1665 (lactam C=O), 1635 (C=C), 1450, 1365, 1270, 1240, 1170, 1060 .
^{1}H-NMR ($CDCl_3$, δ , ppm): 11.29 (sharper broad s, 1H, lactam NH), 10.32 (broad s, 1H, pyrrole NH), 7.28 (s, 5H, Ar-H), 6.12 (s, 1H, =CH-), 5.85 (q, 1H, -CH(CH_3)-O-), 2.9 - 2.2 (m, 6H, -CH_2-CH_3, -(CH_2)$_2$-COO-), 2.35 (s, 3H, -$CH_3$$^{3'}$ or $^{5'}$), 2.11 (s, 3H, -$CH_3$$^{5'}$or $^{3'}$), 1.93 (s, 3H, -CH_3^{3}), 1.51 (d, 3H, -CH(CH_3)-O-), 1.18 (t, 3H, -CH_2-CH_3).
UV-Vis, λ_{max}, nm (ε)· CH_2Cl_2, 402 (50,000); DMSO, 410 (50,000).

High resolution electron impact mass spectrum (HREIMS), m/z (rel. intens.)· 406.2262 (100) [M^{+}·, calculated for $C_{25}H_{30}N_2O_3$ 406.22563], 302.1628 (45), 301.1539 (40), 203.1483 (38), 213.1031 (7), 105.0703 (30) amu.
Elemental analysis (EA): Calc. C 73.86 H 7.44 N 6.89; Found C 73.72 H 7.61 N 7.17

XBR-(R)-sec-Phenethyl Ester, 2. Prepared according to the general procedure, from XBR and (R)-sec-phenethyl alcohol with 72% yield (M.p.= 149- 151°C). All chromatographic and spectroscopic data as per the (rac)- ester 1.
EA: Calc. C 73.86 H 7.44 N 6.89; Found C 73.42 H 7.57 N 7.22
CD· $CHCl_3$, bisignate, λ_{max} = 445 nm (+), 400 (-); DMSO, monosignate, λ_{max} = 410 nm (-). Dependence of CD with concentration is as follows:

Solvent	[*2*], *M*	*Cell length (cm)*	$\Delta\varepsilon_{445}$	$\Delta\varepsilon_{400}$	$(\Sigma(\Delta\varepsilon))$
$CHCl_3$	*5 x 10^{-3}*	*0.01*	*1.4*	*-2.9*	*4.3*
$CHCl_3$	*5 x 10^{-4}*	*0.1*	*1.55*	*-3.45*	*5.0*
$CHCl_3$	*5 x 10^{-5}*	*1*	*1.6*	*-4.15*	*5.75*
$CHCl_3$	*5 x 10^{-6}*	*10*	*0.8*	*-1.45*	*2 25*
DMSO	*5 x 10^{-3}*	*0.01*		*-1.38*	*1.38*

XBR-(S)-sec-Phenethyl Ester, 3. Prepared according to the general procedure, from XBR and (S)-sec-phenethyl alcohol with 85% yield (M.p.= 149- 151°C).All chromatographic and spectroscopic data as per the (rac)- ester 1.
EA. Calc. C 73.86 H 7.44 N 6.89, Found C 73.40 H 7.58 N 7.20
The CD results are basically equal -with opposite signs- to those of the (R)-enantiomer. The values obtained in the CD-concentration dependence experiments are as follows

Solvent	[*2*], *M*	*Cell length (cm)*	$\Delta\varepsilon_{445}$	$\Delta\varepsilon_{400}$	$(\Sigma(\Delta\varepsilon))$
$CHCl_3$	*5 x 10^{-3}*	*0.01*	*-1.3*	*2.3*	*3.6*
$CHCl_3$	*5 x 10^{-4}*	*0 1*	*-1.6*	*2.4*	*4.0*
$CHCl_3$	*5 x 10^{-5}*	*1*	*-2.0*	*2.6*	*4.6*
$CHCl_3$	*5 x 10^{-6}*	*10*	*-1.05*	*2 25*	*3.3*
DMSO	*5 x 10^{-3}*	*0.01*		*0 75*	*0.75*

XBR-(rac)-sec-Butyl Ester, 4. Prepared according to the general procedure, from XBR and (rac)-sec-butyl alcohol with 93% yield (M.p.= 182- 184°C). HPLC (see above for conditions)· R_t= 5.8 min.
IR (KBr, cm^{-1}) 3350 (NH), 2910, 1730 (ester C=O), 1675 (lactam C=O), 1630 (C=C), 1450, 1365, 1270, 1170 .
^{1}H-NMR ($CDCl_3$, δ , ppm) 11.24 (sharper br. s, 1H, lactam NH), 10.33 (br. s, 1H, pyrrole NH), 6.13 (s, 1H, =CH-), 4.82 (q, 1H, -O—CH(CH_3)-), 2.9 - 2.2 (m, 6H, -CH_2-CH_3, -

(CH_2)$_2$-COO-), 2.41 (s, 3H, -$CH_3^{3'\text{ or }5'}$), 2.14 (s, 3H, -$CH_3^{5'\text{or }3'}$), 1.94 (s, 3H, -CH_3^3), 1.7 - 1.4 (m, 5H, -O--CH(CH_3)-CH_2-CH_3), 1.17 (t, 3H, -CH_2-CH_3), 0.87 (t, 3H, -O-CH(CH_3)-CH_2-CH_3).
UV-Vis; λ_{max}, nm (ε): CH_2Cl_2, 402 (50,000), DMSO, 410 (50,000).
HREIMS, m/z (rel. intens.): 358.2252 (100) [$M^{+\cdot}$, calculated for $C_{21}H_{30}N_2O_3$ 358.22563], 302.1627 (20), 285.1595 (15), 243.1492 (42) amu.
EA: Calc. C 70.36 H 8.43 N 7.81, Found C 70.01 H 8.59 N 8.13

XBR-(R)-sec-Butyl Ester, 5. Prepared according to the general procedure, from XBR and (R)-sec-butyl alcohol with 90% yield (M.p.= 182- 184°C). All chromatographic and spectroscopic data as per the (rac)- ester 4.
EA: Calc. C 70.36 H 8.43 N 7.81; Found C 70.25 H 8.56 N 8.09

XBR-(S)-sec-Butyl Ester, 6. Prepared according to the general procedure, from XBR and (S)-sec-butyl alcohol with 91% yield (M.p.= 182- 184°C). All chromatographic and spectroscopic data as per the (rac)- ester 4.
EA: Calc. C 70.36 H 8.43 N 7.81, Found C 70.27 H 8.46 N 8.08

XBR-(rac)-2-Octyl Ester, 7. Prepared according to the general procedure, from XBR and (rac)-2-octyl alcohol with 85% yield (M.p.= 111- 113°C). HPLC (see above for conditions) R_t= 9.7 min. Due to the characteristic smell of the starting alcohol, the presence of even traces of it can be monitored in this way.
IR (KBr, cm^{-1}): 3350 (NH), 2920, 2855, 1730 (ester C=O), 1675 (lactam C=O), 1630 (C=C), 1450, 1365, 1270, 1175 .
^{1}H-NMR ($CDCl_3$, δ , ppm): 11.28 (sharper br. s, 1H, lactam NH), 10.36 (br. s, 1H, pyrrole NH), 6.12 (s, 1H, =CH-), 4.87 (q, 1H, -O-CH(CH_3)-), 2.9 - 2.2 (m, 6H, -CH_2-CH_3, -(CH_2)$_2$-COO-, 2.41 (s, 3H, -$CH_3^{3'\text{ or }5'}$), 2.14 (s, 3H, -$CH_3^{5'\text{or }3'}$), 1.94 (s, 3H, -CH_3^3), 1.7 - 0.85 (m, 19H, -O-CH(CH_3)-(CH_2)$_5$-CH_3, -CH_2-CH_3).
UV-Vis; λ_{max}, nm (ε): CH_2Cl_2, 402 (50,000); DMSO, 410 (50,000).
HREIMS, m/z (rel. intens.). 414.2876 (100) [$M^{+\cdot}$, calculated for $C_{25}H_{38}N_2O_3$ 414.28823], 383.2564 (4), 302.1628 (22), 243.1499 (30) amu.
EA Calc. C 72.43 H 9.24 N 6.76, Found C 72.22 H 9.35 N 7.06

XBR-(R)-2-Octyl Ester, 8. Prepared according to the general procedure, from XBR and (R)-2-octyl alcohol with 85% yield (M.p.= 111- 113°C). All chromatographic and spectroscopic data as per the (rac)- ester 7.
EA. Calc. C 72.43 H 9.24 N 6.76; Found C 72.34 H 9.25 N 7.07

XBR-(S)-2-Octyl Ester, 9. Prepared according to the general procedure, from XBR and (S)-2-octyl alcohol with 85% yield (M.p.= 111- 113°C). All chromatographic and spectroscopic

data as per the (rac)- ester 7.
EA. Calc. C 72.43 H 9.24 N 6.76; Found C 72.67 H 9.29 N 7.04

XBR-MPEG Monoester, 10. The procedure is basically the same as that described above for the synthesis of XBR esters 1 to 9.

The amounts of reagents used are: XBR (0.16 meq, 48.3 mg), MPEG-1900 (0.10 meq, 190 mg), DCCI (0.18 meq, 37 mg), DMAP (0.03 meq, 3.8 mg) in CH_2Cl_2 (3 ml).

The yellow-brownish solid isolated after filtering out the precipitated urea is washed with acetone (3 x 0.5 ml) and the additional urea precipitated after each washing filtered off. The residue is dissolved in CH_2Cl_2 (1 ml) in an ice bath and, with magnetic stirring, 5 ml of cold ether are added. After ca. 5 min, the precipitate is vacuum filtered, washed with cold ether (2 x 5 ml), dried and crystallized from EtOH (10 ml) to yield 204 mg (90% based on MPEG) of a yellow-greenish solid, pure by HPLC (see above for conditions). R_t= 3.1 min. M.p.= 50- 52°C.
IR (KBr, cm^{-1}): 3340 (NH), 2890 (MPEG), 1735 (ester C=O), 1665 (lactam C=O), 1635 (C=C), 1470, 1345, 1285, 1245, 1150, 1115 (MPEG), 1065, 970, 850.
^{1}H-NMR ($CDCl_3$, δ , ppm): 10.8 (sharper broad s, 1H, lactam NH), 10.2 (broad s, 1H, pyrrole NH), 6.1 (s, 1H, =CH-), 3.6 (s, ca. 90 H, polymeric $-CH_2-$), 3.3 (s, 3H, polymeric $-O-CH_3$), 2.9 - 2.3 (m, 6H, $-CH_2-CH_3$, $-(CH_2)_2-COO-$), 2.3 (s, 3H, $-CH_3$ 3' or 5'), 2.1 (s, 3H, $-CH_3$ 5' or 3'), 1.95 (s, 3H, $-CH_3$ 3), 1.2 (t, 3H, $-CH_2-CH_3$).
UV-Vis; λ_{max}, nm (ε) H_2O, 415 (30,000), 276 (shoulder, E isomer?).

XBR-)$_2$-PEG Diester, 11. The procedure is identical to that described for the XBR-MPEG monoester 10. The amounts of reagents used were XBR (0.22 meq, 66.4 mg), PEG-2000 (0.1 meq, 100 mg), DCCI (0.25 meq, 51.5 mg), DMAP (0.04 meq, 5.0 mg) in CH_2Cl_2 (3 ml).

After 24 h at room temperature, reverse phase HPLC (see above for conditions) showed esterification of 44% of starting XBR, corresponding to 97% yield based on starting PEG. R_t= 3.6 min. The title ester (122 mg) was isolated as indicated above for the monoester 10. M.p.= 50- 52°C.
IR (KBr, cm^{-1}): Similar to that of the parent monoester 10, with chromophore bands more intense relative to those of the polymer.
^{1}H-NMR ($CDCl_3$, δ , ppm): Similar to that of the parent monoester 10, except for the signal at 3.3 (s, 3H, polymeric $-O-CH_3$) which is absent. Expectedly, integration of the chromophore protons, relative to the polymer is twice as much as in 10.
UV-Vis; λ_{max}, nm (ε): H_2O, 412 (60,000), 275 (shoulder, E isomer?).

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