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POLYMER BOUND PYRROLE COMPOUNDS- II[1]. SYNTHESIS AND PROPERTIES OF POLYSTYRENE-BOUND LINEAR TETRAPYRROLE PIGMENTS AND RELATED 5(1H)-PYRROMETHENONES

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Abstract- *The title chromophores have been reversibly and covalently attached to differently functionalized polystyrenes including the α-[(4-bromomethyl-3-nitrobenzamido)benzyl]poly(styrene-co-divinylbenzene), and its desnitro derivative . The influence on the reaction of the nature and concentration of chromophore and of the resin is discussed, as it is the effect of the functionalization degree of the resin and of the reaction time, among others. The above data, together with studies on the nature of detachment products, and on the anchoring to a monomeric model of the above polystyrenes, afforded mechanistic information on these processes. Different approaches to obtain spectroscopic data about the anchored pigments, including their solubilization, are described. Finally, an example of a "chemical" way to derive information on the structure and mobility of the supported chromophores is given.*

INTRODUCTION*

Linear tetrapyrroles are found in nature either as catabolic products forming complexes with proteins or as the covalently-bound chromophores of biliproteins[2]. These are biologically relevant[3-5] compounds with remarkable structural features which include phycocyanin, phytochrome and bilirubin (BR) among others. In the past, most investigations have centered on the structural features and reactivity of the isolated tetrapyrroles in solution[6]. However, this is a simplification which can, in extreme instances, even invalidate the obtained results when applied to the natural systems. On the other hand, the interactions between the chromophore and protein in natural systems are just now being revealed[7]. This is due to their complexity and instability and, especially in plants, to the low concentration of active species.

A better model for the natural system consists of the bile pigment linked to a previously functionalized polymer support. Such a model shall permit the study of the influence of macromolecules on the pigment stereochemical and electronic structural properties and their energy-transfer mechanisms.

We have recently reported[1] on the covalent binding of bile pigments to an insoluble support: both BR and BV, via their cesium salts, were covalently attached to a polystyrene matrix (the so-called Nbb-resin), and then detached from the resulting products by alkaline methanol treatment. Polystyrenes have been used extensively in the solid-phase synthesis of peptides[8]; furthermore,

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* *Abreviations: BR, bilirubin IX-α; BV, biliverdin IX-α; XBR, xanthobilirubinic Acid; XBRNMe, xanthobilirubinic Acid N'-CH_3 substituted; MBV, mesobiliverdin XIII-α; the terminations ME and DME indicate methyl ester and dimethyl ester respectively; the terminations Cs and Cs_2 indicate the mono- and dicesium salts, the terminations NBu_4 and $(NBu_4)_2$ indicate the mono- and di(tetra-n-butylammonium) salts; bromomethyl-Nbb or alternatively Br-CH_2-Nbb stands for α-[(4-bromomethyl-3-nitrobenzamido)benzyl]-poly(styrene-co-divinylbenzene), (7a).*

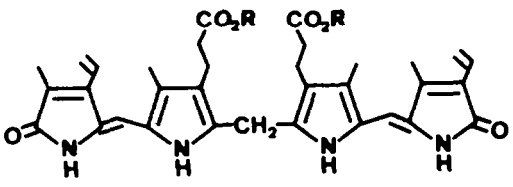
they will allow in the future to insert one or more aminoacids between the resin and the chromophore, thus improving their resembling of the natural systems. As outlined in our previous communication[1], the well known Merrifield chloromethylated polystyrene was found to give too low yields of anchoring. The loading in pigment to the chloromethylated polystyrene achieved by other authors working with mesoporphyrin[9] or with heme[10], must be much lower than ours.
Structural knowledge of the insoluble, polymer-supported chromophores can be derived from spectroscopic measurements, including IR, UV-Vis, gel-phase ^{13}C-NMR spectroscopy[11] and luminescence[12] among others. Complementary information can be derived chemically: one possibility is to investigate the detached chromophore; as will be seen, the base-MeOH detachment[13] gives transesterification products which are soluble and can thus be identified. Several structural details cannot be investigated in this way, and in these cases other processes must be designed.
In the following pages, we want to demonstrate the validity of studying the polymer-attached pyrrole pigments, as a way to investigate an isolated pigment molecule in the condensed phase as well as the possible interactions between one such molecule and the polymer, and even interchromophoric interactions between pigment molecules.

In the present paper, we report on the optimization of the anchoring of pigments containing acid lateral chains to the insoluble bromomethyl-Nbb resin 7a according to ref.[1], and on the extension of this reaction to other chromophores, including verdins, and model pyrromethenones, as well as to other supports (such as 7b and 7c), in an attempt to elucidate the factors determining both the anchoring and deanchoring. We also discuss the "solubilization" of the anchored pigments in organic solvents; and describe their UV-Vis spectra. Finally, we report on the use of the extension of the DDQ/TFA catalyzed self-condensation of 5'-CH_3-pyrromethenones to biliverdins[14] as a measure of the proximity and mobility of the pyrromethenone units within the polymer matrix.

RESULTS AND DISCUSSION

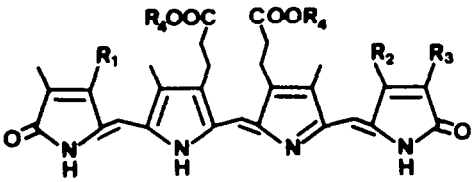
Reversible Anchoring of Pigments to the Resins 7a and 7b, and to the Monomer 7c:

The synthesis of all compounds previously reported is briefly discussed in the Experimental Part. The XBR-N'-CH_3 derivatives (4a-c) have been obtained for the first time following a similar strategy to that reported for XBR itself[15] but using the respective N'-CH_3 pyrrole 6d in the condensation step (see Experimental).
The so-called desnitro-bromomethyl-Nbb resin (7b) has been prepared identically to 7a although omitting the nitration of the intermediate 4-bromomethylbenzoic acid.
All pigments of this work have been anchored through their propionic acid side chains. The process of anchoring involves neutralization of DMF suspensions of the pigments with aqueous Cs_2CO_3 to give the corresponding cesium salts, which are then used in the attachment reactions. This procedure is a modification of that used by Gisin in the solid-phase peptide synthesis[16]. The resulting cesium salts have been identified spectroscopically. In the case of BR IX-α, the base treatment and the attachment are accompanied by different degrees of scrambling (10-25% of each symmetrical isomer are produced). Scrambling of BR IX-α in similar conditions has been reported recently[17] and the reaction is inhibited among others by thiourea[18]. Unfortunately, in the presence of thiourea, binding of BR disalt to 7a in DMF proceeded with notably lower yield.
Following Gisin's procedure, we have anchored BR IX-α (1a), BV IX-α (2a), MBV XIII-α (2d), XBR (3a), and XBR-N'-CH_3 (4a), to the bromomethyl-Nbb resin (7a); XBR (3a) has also been attached to the desnitro resin (7b) and both 1a and 3a to monomeric Br-CH_2-Nbb (7c). The process involves treatment of the insoluble resin or soluble monomer with a DMF solution of the pigment cesium salt at RT for periods oscillating between 3 and 24 hours. The attachment yields have in most cases been higher than 70%, calculated from the weight gain. The binding to the resin is confirmed by a benzyl ester band near 1735 cm^{-1} in the IR spectrum of the product.
Attachment of BR IX-α to 7a has also been achieved via its bis-(tetra-n-butylammonium) salt[17], using CH_2Cl_2 as the solvent. The general effect of the counter-ion in the esterification of BR (i.e., mono- vs. diesterification) is more extensively discussed below.



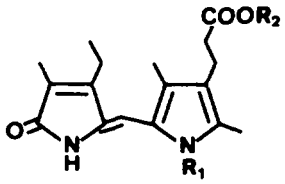
1

1	R
a	H
b	Cs
c	CH_3
d	NBu_4



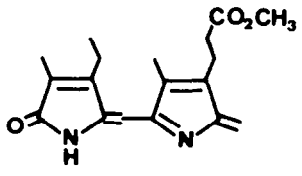
2

2	R_1	R_2	R_3	R_4
a	$CH=CH_2$	CH_3	$CH=CH_2$	H
b	$CH=CH_2$	CH_3	$CH=CH_2$	Cs
c	$CH=CH_2$	CH_3	$CH=CH_2$	CH_3
d	CH_2CH_3	CH_2CH_3	CH_3	H
e	CH_2CH_3	CH_2CH_3	CH_3	Cs
f	CH_2CH_3	CH_2CH_3	CH_3	CH_3

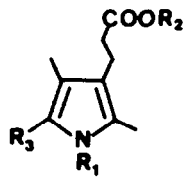


3 and 4

	R_1	R_2
3a	H	H
3b	H	Cs
3c	H	CH_3
4a	CH_3	H
4b	CH_3	Cs
4c	CH_3	CH_3

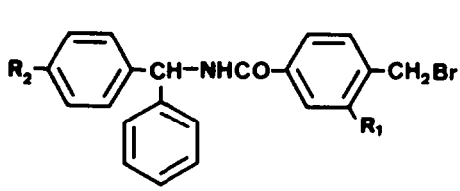


5



6

6	R_1	R_2	R_3
a	H	C_2H_5	$CO_2C_2H_5$
b	CH_3	C_2H_5	$CO_2C_2H_5$
c	CH_3	H	CO_2H
d	CH_3	H	H



7

7	R_1	R_2
a	NO_2	Ⓟ*
b	H	Ⓟ*
c	NO_2	H

Ⓟ* stands for poly(styrene-co-divinylbenzene)

Optimization of the Process of Anchoring. The optimization experiments performed are summarized in Table 1. From them, the following conclusions could be drawn: 1) verdins give higher yields of attachment than rubins; 2) the extension of anchoring increases with the reaction time to a maximum of 24 h, when the reaction is driven to the maximum achievable under the given conditions; and 3) attachment increases with the absolute concentration of chromophore and of anchoring sites (i.e., the degree of functionalization, F, of the resin) as well as with the ratio of equivalents of chromophore to resin. These results, together with others discussed below, suggest an S_N2 mechanism, but it falls outside the scope of this work to unequivocally elucidate that.

The repeated larger yield in the anchoring of verdins as compared to rubins is in apparent contradiction with the expected major conformational flexibility of rubins (where the central bridge (C10) is saturated). However, rubin cesium salts in DMF are strongly intramolecularly hydrogen bonded and prefer a folded, rigid conformation in which each carboxylate is strongly hydrogen bonded to the NH's of the other half of the molecule. As a result, their accessibility before substitution at the polymeric benzylic site is hindered. Verdin cesium salts, instead, probably have nearly planar, helical conformations, in which the propionic acid chains are more flexible and free to react. We have established spectroscopically the hydrogen bonded conformations of cesium salts of BR and XBR, and, for the first, our results agree with those of Lightner et al for BR bis(tetra-n-butylammonium) salt[17] .

One might feel tempted to further explain the lower yield for bilirubin anchoring in terms of an intermolecularly hydrogen bonded conformation of polymer-bound BR which would increase its reticulation and difficultate the binding of subsequent pigment units. Indeed, a hydrogen bonded conformation has been reported for BRDME (1c) in solution[19], and we have spectroscopically confirmed hydrogen bond formation in BR-CH_2-Nbb[11]. However, the same studies do not show a direct relationship between swelling and hydrogen bond formation[11].

The Effect of the Counter-ion. Mono- vs. Diesterification[20]. It has recently been found[20] that in a related nucleophilic substitution, BR bis-tetra-n-butylammonium salt in acetone or CH_2Cl_2 rapidly reacts at RT with excesses of alkyl iodides and other alkyl derivatives to selectively give with high yields the respective monoesters. While in solution monoesterification predominates, under solid phase conditions one should expect dianchoring to be almost the exclusive process. In order to establish the extension of intramolecular reaction in heterogeneous phase, we compared the products from the reactions of BR dicesium salt in DMF, and of BR bis-tetra-n-butylammonium salt in CH_2Cl_2 with 1) CH_3I, 2) monomeric Br-CH_2-Nbb (7c), and 3) Br-CH_2-Nbb (7a). Several results (Table 2) deserve a comment: 1) the percentage of unreacted BR disalt markedly increases in going from CH_3I to monomeric Br-CH_2-Nbb (to be synthetically interesting, reaction with the last should be extended to approximately 1 h); 2) the Cs cation gives higher esterification yields than tetra-n-butylammonium; 3) for a given counter-ion, the relative percentage of monoester decreases in going from CH_3I to monomeric Br-CH_2-Nbb and to polymeric Br-CH_2-Nbb, and in this respect, the ammonium cation clearly is more selective than Cs towards monoesterification. Decrease in selectivity in the reaction in front of the polymer may be due to longer reaction times (the relative percentage of monoester in the reaction of $BRCs_2$ with CH_3I decreases at longer reaction periods). However, diattachment of linear tetrapyrroles to polymeric Br-CH_2-Nbb is the major process even after only 3 h reaction, and this must be due to a cooperative effect by the polymer: once BR is bound to the resin through one propionic acid chain, it is much easier for the second carboxylate to find a point in the resin where to anchor. This is important in relation with the controversy of wether a resin is capable or not of providing effective site isolation of a group (the so-called "infinite dilution" effect)[21]. Our results are thus contrary to what Lezznoff and others[22] have proposed in the past; i.e., that the solid-phase work has considerable potential for allowing the attachment of symmetrycal polyfunctional compounds by one group only. As a matter of fact, it is quite accepted nowadays that the "infinite dilution" effect only occurs to an important extension in highly reticulated polymers (i.e., 20% or higher), while site-site interactions are important in polymers of lower crosslinking[23].

Table 1: Yields of Anchoring of BR (1a), BV (2a) and MBV (2d) to Bromomethyl-Nbb Resins (7a) of functionality[a] F= 0.65 and 1.05

Assay	Chromophore	[Chrom] meq.ml^{-1}	Starting functionality (meq.g^{-1})	[Chrom]/ [Resin]	Reaction Time (h)	Anchoring Yield (%)[b]	Final functionality (meq.g^{-1})
1	1a	0.1	0.65	1.4	24	54	0.32
2	1a	0.1	0.65	1.5	13	40	0.24
3	1a	0.1	1.05	1.0	10	72	0.61
4	1a	0.04	1.05	1.0	24	76	0.68
5	1a	0.09	1.05	1.0	24	73	0.66
6	1a	0.05	1.05	0.5	24	75	0.41
7	1a	0.02	1.05	0.5	24	60	0.29
8	2a	0.05	0.65	1.5	24	90	0.54
9	2a	0.03	0.65	1.5	6	60	0.36
10	2a	0.03	0.65	1.5	3	55	0.32
11	2a	0.05	1.05	1.0	24	61	0.58
12	2a	0.03	1.05	1.0	6	55	0.45
13	2a	0.03	1.05	1.0	3	25	0.20
14	2a	0.08	1.05	1.0	13	80	0.65
15	2d	0.05	1.05	1.0	24	64	0.60

[a] Expresses meq of a given reactive group (for example Br-CH_2-) per g of solid. [b] Calculated always with respect to the least abundant component. It indicates percentage of starting binding sites which have been esterified, except in Assays 6 and 7 where it corresponds to the percentage of starting rubin salt attached. In the last cases, it is better to compare the Final functionality values.

Table 2: Final Products from the Reaction of $BRCs_2$ (1b) and $BR(NBu_4)_2$ (1d) with Different Substrates

Reaction	[BR Disalt]/ [Substrate]	Unreacted BR Disalt (%)[a]	BR Monoester (%)[a]	BR Diester (%)[a]
$BRCs_2$ + CH_3I[b]	Excess CH_3I	19	54	27
$BRCs_2$ +Monomer (7c)[b]	1.0	70	15	15
$BRCs_2$ +Polymer (7a)[c,d]	1.0	25	10	65
$BR(NBu_4)_2$ +CH_3I[e]	Excess CH_3I	50	45	5
$BR(NBu_4)_2$ +Monomer (7c)[e]	1.0	80	20	-
$BR(NBu_4)_2$ +Polymer (7a)[d,f]	1.0	30	25	45

[a] Percentages given are within a + 5% error margin; [b] In DMF, 10 min, RT; [c] In DMF, 24 h, RT; [d] 7a has a functionality, F= 1.05; [e] In CH_2Cl_2, 10 min, RT; [f] In CH_2Cl_2, 24 h, RT.

Deanchoring of Pigments from the Resins 7a and 7b, and from the Monomer 7c. All pigments anchored throughout this work have subsequently been detached from their polymeric derivatives by alkaline MeOH treatment, according to the procedure reported[13] for the deanchoring of peptides from a chloromethyl- and a 2-hydroxyethylsulphonylmethyl- polystyrenes. The process involves breaf treatment of the insoluble attached pigment with a dioxane:MeOH:4N-NaOH (30:9:1) solution at RT. The yields of detachment are close to completeness and the pigments recovered almost exclusively as the (di)methyl esters of the original (di)acids (BR gives ca. 10% of monoesters). In a blank experiment, XBR free acid treated with the same alkaline MeOH was recovered unreacted.

Obtention of only methyl esters implies that a mechanism of nucleophilic substitution by either of the two bases present in the reaction medium (HO^- and MeO^-) does not operate here since, if this were the case, pigments would be recovered as the free acids. It also agrees with a transesterification mechanism ($B_{AC}2$)[24] and means that only MeO^- is capable of reacting. On the contrary, in the hydrolysis of XBR bound to the monomer 7c, this selectivity in favour of MeO^- decreases and a mixture of acid and ester containing near a 25% of acid is formed.

The important point here is the marked increase in MeO^- selectivity in going from homogeneous to heterogeneous conditions. It is clear that the increase in selectivity cannot be related only to the bases, but must involve the set of polymer and attached species.

The fact that pigment diacids are always detached as dimethyl esters, indicates that all were originally doubly anchored to the resin through their two propionic lateral chains as a consequence of the mobility of the polymeric backbone during the anchoring process. Furthermore, any future attempt to achieve the synthetically attractive monoattachment of diacids, must utilize a much poorly functionalized resin: i.e., one with an F of 0.3 or less.

Since BR undergoes free-radical isomerization during the process of binding, a completely scrambled mixture of BR dimethyl esters is obtained in the detachment of polymer-bound BR IX-α.

The Effect of the Nitro Group. The bromomethyl-Nbb resin 7a was first reported[25] as a superior alternative to the chloromethylated polystyrene for the solid-supported synthesis of peptides. However, in the study of the photoprocesses of the polymer supported bile pigments, the presence of an extra photo-sensitive group in our compounds might fail to be an advantage[26]; therefore, the synthetic necessity for this nitro group needed to be investigated. With this objective in mind, we compared the attachment of XBR (3a) to -and subsequent detachment from- bromomethyl-Nbb resins having (as in 7a) or not (as in 7b) the nitro group. The results are summarized in Table 3. They indicate that the influence of the nitro group is only slight and it favours the anchoring, but difficultates the detachment. The larger yield obtained in the anchoring to the nitrated resin is also in agreement with an S_N2 mechanism[27]. On the other hand, attachment probably implicates electromeric and electrostatic effects or even a neighbouring group effect by the ortho-nitro group, and we are working in the confirmation of this.

The detachment result , which might be due to the steric hindrance by the ortho-nitro group, is in agreement with our proposal that a $B_{AC}2$ -and not an S_N- mechanism operates during the deanchoring. The conclusion is, in future investigations involving photoprocesses, a resin lacking the nitro group, such as 7b can be more convenient since this will not effect the synthetic yields.

Solid Phase versus Homogeneous Reactions. The binding product of BR to the monomer 7c -after much shorter reaction times than in the anchoring to the parent polymer 7a- consists of mixtures containing percentages of monoesters equal or higher than these of diesters (Table 2). On the contrary, in the binding to polymeric 7a, diesters are almost the only product. This demonstrates the existence of an "hiperentropic" effect in the last system.

We have also compared the anchoring of XBR to the resin 7a and to its monomeric model 7c. Under the best conditions assayed (Table 3), attachment to 7a takes place with a 91% yield after 24 hours of reaction, while binding to soluble 7c occurs to near completeness after only 2 hours. This result, which goes in the same direction of what is found in the anchoring of BR to 7a and to 7c (Table 2), shows that while in an homogeneous medium there are no restrictions to the

Table 3: Yields of Anchoring and Deanchoring of XBR (3a) and XBR-NMe (4a) to the Supports 7a (with Nitro Group), 7b (without Nitro Group) and 7c (Monomeric)

Support	Chromophore	[Chromoph], meq.ml^{-1}	Starting functionality (meq.g^{-1})	[Chromoph]/[Resin]	Reaction Time (h)	Anchoring Yield (%)	Final functionality (meq.g^{-1})	Deanchoring Yield (%)
7a	3a	0.04	1.05	1.0	24	91[a]	0.81	81
7b	3a	0.035	0.95	1.1	24	74	0.75	99
7c	3a	0.017	-	1.0	2	99	-	99
7a	4a	0.035	1.05	1.0	24	92	0.81	99

[a] Average of 3 runs

Table 5: DDQ/TFA Catalyzed Self Condensation of XBRME (3c) and of XBR Bound to the Resin 7a[a]

Assay	Phase	Description	[Pyrromethenone], mol.l^{-1}	[DDQ], mol.l^{-1}	%TFA	[DDQ]/[Pyrromethenone]	% Starting Pyrromethenone Condensed (A)[b]	% Starting Pyrromethenone Oxidized (B)[c]	B/A[d]
1	Homogeneous	Our results using Falk's conditions[e,f]	6.7	8	3.4	1.2	36	ca.10	0.28
2	Homogeneous	Falk's conditions adapted to solid-phase requirements	9.6	11	5.7	1.2	30[g]	20	0.67
3	Homogeneous	Effect of an excess DDQ	11	23	16.7[h]	2.1	12	54[i]	4.5
4	Heterogeneous	Self-condensation of XBRCH$_2$Nbb[j]	12.5[k]	13.7	6.25	1.1	ca.40	ca.15	2.67
5	Heterogeneous	Self-condensation with excess DDQ	14.7[k]	29	16.7	2	26	45	1.7

[a] For the rest of experimental conditions, see Experimental. [b] The yield of resulting verdin is twice this amount. [c] The yield of aldehyde coincides with this value. [d] The larger this value, the least favoured is the self-condensation process. [e] Reference 14. Falk reports A= 30 and gives no data on B. [f] Best result of several runs. [g] The yield of tetrapyrrole is 60% (40% rubin + 20% verdin). [h] A similar % to that of Assay 2 should have been used. [i] This percentage includes the aldehyde and a species not completely identified, probably the respective alcohol or ketal. [j] A blank assay lacking DDQ affords unmodified material. [k] Concentration of pyrromethenone calculated from the functionality of the resin in that compound.

approaching of reagents, under heterogeneous conditions, the solubilized species must reach all reactive sites of the polymeric matrix in order to completely bind, and the difficulty for this to happen under the given conditions, will depend on the nature (i.e. hydrogen bonding, counter-ion, lipophilicity, size and shape ...) of the soluble species; it will also depend on the solvent (i.e., its polymer swelling capability, hydrogen bonding breaking and solvating properties, etc.), and obviously on the polymer itself: mainly its mobility (i.e., crosslinking), but also functionalization degree, lipophilicity, and others.

The complete hydrolysis of both XBR- and BR-monomeric nitrobenzyl esters was also similar to that discussed for 7a-b.

Finally, neither the attachment nor the detachment processes were found to give significant amounts of byproducts; the last, if in sufficiently low concentrations, might have been missed in the analytical determinations upon their insoluble partners.

UV-Vis Spectroscopy of the Polymer-supported Pigments:

We have assayed two different approaches with diverse fortune (Table 4): the first concerns the spectroscopy of KBr pellets of the insoluble attached pigments. In spite of its relative experimental complexity, the approach permitted the recording of spectra of all chromophores assayed. However, the maxima obtained (Table 4) are close to the values found with KBr pellets of methyl esters -or free acids- of the same chromophores. This is the limitation of this approach: the dispersion of the supported chromophore in KBr will not permit to conclude to what extent the shifts in the spectra, as compared to these in solution, are due to the macromolecule.

Table 4: UV-Vis Absorption Spectra (up from 370 nm) of Bile Pigments and their Polymer Anchored Derivatives in Different Media (λ_{max} in nm)[a]

Compound	$CHCl_3$/1% EtOH	MeOH	KBr
Manually grinded BR bound to the polymer 7a[b]	420, 465[c,d]	-	-
Mechanically grinded BR bound to the polymer 7a[e]	395, 435 (sh)	385, 425 (sh)[f]	420, 480 (sh)
BR bound to the monomer 7c	399, 440 (sh)[g]	-	-
BR (1a)	454	452[h]	420, 480 (sh)
$BRCs_2$ (1b)	460[i,j]	-	-
BRDME (1c)	400[k]	453[f,l]	415,470 (sh)
Manually grinded BV bound to the polymer 7a[b]	400, 720[d,m]	-	-
Mechanically grinded BV bound to the polymer 7a[e]	378, 618[n]	-	-
BV (2a)	380, 658	376, 666	374, 656
$BVCs_2$ (2b)	381, 730[i]	-	-
BVDME (2c)	378, 653	380, 660	375, 640
XBR bound to the polymer 7a[o]	400	405[p]	-
XBR bound to the polymer 7b[o]	400	410	-
XBR bound to the monomer 7c	399[q]	411	-
XBR (3a)	-	416	-
XBRCs (3b)	408[q]	418[p]	-
XBRME (3c)	405[q]	414	399
XBRNMe bound to the polymer 7a	385[d]	-	-
XBRNMe (4a)	394	-	-
XBRNMeCs (4b)	409	-	-
XBRNMeME (4c)	393	401[p]	-

[a] Spectra of most attached pigments are recorded against solutions of the same concentration of 7a with a functionality F= 1.05. [b] Relatively large particle size; i.e., larger than 0.45 μm. [c] Flat, broad band. [d] Dry CH_2Cl_2. [e] Particle size smaller than 0.45 μm. [f] In DMSO, this work. [g] Approximately 1:1 mixture of BR mono- and diester in dry CH_2Cl_2. [h] In 0.2% NH_3/MeOH, acc. to Bonnett[28]. [i] Wet DMF. [j] $BRCs_2$ + Thiourea has λ_{max} (DMF)= 456 nm. [k] 397 and 445 nm (sh) in 2-MTHF, acc. to Schaffner[3]. [l] 448 and 408 nm (sh) in EtOH[3]. [m] Relative intensities of the two bands is 1:1. [n] Very flat, broad band which difficultates localization of the λmax. [o] Sample manually grinded and filtered through paper. [p] EtOH. [q] Dry $CHCl_3$.

The spectrum of BR anchored to 7a in KBr has a maximum near 420 nm and a shoulder near 475 nm. These values almost exactly reproduce the λ_{max}^{exc} values reported[3] for EtOH and 2-MTHF solutions of BRDME at 77 K. Freezing the solution has the same result upon the spectrum of the chromophore as preparing a solid dispersion of it. This confirms the unsuitability of KBr pellets of polymer bound pigments in the study of their photoisomerization, a process which has been demonstrated to be inhibited by the viscosity of the environment[7a] and by low temperatures[29].

In our second approach, UV-Vis spectra of suspensions of bound pigments were attempted. However, only when the insoluble beads are very well swollen by the solvent and form a partially transparent gel, could the spectra be recorded. Better results are obtained if the beads of bound pigments are first manually grinded, and then a suspension of the powder is eventually filtered. Interestingly, large-particle samples of BR attached to polymer 7a (prepared by manual grinding) in CH_2Cl_2 "solution" also have maxima near 420 and 465 (sh) nm (no reference can be made to ε values since the real concentration of chromophore in solution is not known; on the other hand, scattering effects are wavelength dependent). In manually grinded linked biliverdins, the 640- 660 nm band of biliverdins in solution blueshifts up to 720 nm (expectedly, they also have different $\varepsilon_{380}/\varepsilon_{650}$ ratios than the corresponding solution forms due to scattering).

A more "complete" solubilization of the polymer supported chromophores was carried out by mechanical grinding of the beads, followed by filtration through a 0.45 μm filter. Unfortunately, for sensitive polymer-bound pigments such as bilirubin, this procedure was always accompanied by some chemical degradation of the attached chromophores, and this can affect the final spectra.

Table 4 shows small size polymer-bound BR to have similar λ_{max} values to those of BRDME in 2-MTHF (footnote k in Table) with the maxima blueshifted about 5-15 nm with respect to 1c. From these results, we believe BR bound to 7a is probably intermolecularly hydrogen bonded or at least it has a conformation permitting this type of interaction. In the spectrum of small size polymer-bound verdins there is not a substantial difference with respect to the spectrum of unbound biliverdins. In summary, the position of the absorption peaks of both, polymer-bound BR and BV depend probably on the particle size of the polymer bound chromophore, deviation from the solution spectrum being more important in samples of large particle size. On the contrary, the two bound pyrromethenones of this work, as well as several polymer attached porphyrins and metalloporphyrins[12] show little band shifts depending on the particle size. They are also poorly sensitive to the medium (KBr or solvent), and show no indication about chromophoric interaction between pyrromethenone and resin. The differences in peak position for polymer-bound rubins and verdins as compared to their soluble methyl esters might be due to different chromophore conformations depending on the bead size, also to aggregation effects as a result of high local concentration of bound chromophores. Indeed, a similar shift is reported for biliverdin in vesicular systems[30]. More work is currently in progress in our laboratory to establish this.

Photoisomerization of Supported Chromophores[31,32]:

The absorption spectrum of the support 7a has a maximum near 254 nm thus difficultating the detection of any possible E isomer or photooxidation products formed during irradiation of i.e. polymer attached Z-XBR[33]. The photodissapearance of the Z isomer can however be detected in the absorbance difference (AD) spectrum from the formation of a negative peak near 400 nm. In the pyrromethenone series, we have found by AD spectroscopy that, while XBRME 3c easily photoisomerizes to its E isomer at all concentrations assayed both in $CHCl_3$ and in EtOH with apparently little difference, when the same chromophore is bound to the monomer 7c, isomerization takes place in EtOH with apparently no restriction, while in $CHCl_3$ the extension of this process is inversely related to the chromophore concentration to a point where photooxidation is the unique observable process (synthesis peak near 320 nm). Apparently, under extreme conditions, inter- and (more probably) intramolecular hydrogen bonding can even prevent photoisomerization, and the same probably occurs in its polymeric parent. Unfortunately, there is not evidence for photooxidation in the last system either, since the expected products appear in a part of the spectrum where the absorption by the polymeric support is also important.

In the bilirubin series, the AD spectrum of an irratiated $CHCl_3$ "solution" of mechanically grinded BR bound to 7a shows a negative peak at ca. 430 nm (420 nm in DMSO). This peak is partially recovered after standing at RT in the dark overnight. Incomplete reversibility suggests some degree of photooxidation. Photooxidation -or any other irreversible process- is confirmed by the impossibility of achieving a photostationary state. On the other hand, evidence for photoisomerization is not clear, since a corresponding positive peak due to the resulting photoisomers is not detected.

Structure and Mobility of Polymer Bound Chromophores:

We have studied the extension of the DDQ/TFA catalyzed self condensation of 5'-CH_3-pyrromethenones[14] as a measure of the proximity and mobility of the pyrromethenone units within the polymer matrix (Table 5). The reaction must be performed in absolute THF, otherwise nucleophilic attack by the solvent upon the intermediate azafulvene 5 will occur[14b]. Addition of H_2O for example is expected to end up giving the 5'-formyl derivative of 3c. These considerations should afford important structural information when applied to polymer supported 3c: the relative percentage of biliverdin and aldehyde should give an idea of the capability of bound pyrromethenone units to interact with each other. Assay 1 shows that, under the conditions described by Falk, a small percentage of the parent 5'-formyl pyrromethenone accompanies the verdin 2f, and this percentage increases when the concentration of all reactants is increased to meet the usual heterogeneous reaction requirements (assay 2). The same effect is produced by an increase in DDQ concentration at a given XBRME dilution as in assay 3. This is due to trapping (oxidation) by the extra DDQ of the intermediate 5 to give the aldehyde pathway. A second reason is at any given moment, the percentage of starting pyrromethenone which is protonated and subsequently oxidized to the intermediate 5 is much higher than under diluted conditions.
In relation with the self-condensation of attached XBR, a first, blank assay (identical to #4 and 5 below, but lacking DDQ; this assay is not reflected in Table 5) gave starting attached pyrromethenone unmodified. In the last two experiments (4 and 5), we carried out the self-condensation reaction of XBR-CH_2-Nbb using respectively a 1.1 and a 2.0 DDQ/Pyrromethenone ratios; the relative percentage of aldehyde obtained in each run is markedly smaller than under similar homogeneous conditions (assays 2 and 3 respectively). Clearly, if the right solvent is used (one in which a lightly reticulated resin is well swollen and has a gel-type behaviour), the attached molecules perform much as in solution, and if a sufficiently high pigment loading is used, then the local concentration of pyrromethenone is in fact higher than under the reported homogeneous conditions (where they fill the whole solution volume); furthermore, the entropy factor affecting the interactions between two hanging groups is smaller than the one affecting the interaction between the two same molecules in homogeneous solution.
It is probably true that there is some restriction by the macromolecule to free pyrromethenone interaction, and this would result in higher relative yield of aldehyde. The final result is the combination of at least the two above, opposite contributions, and the net sign will depend on the relative importance of each. Under our conditions, enhancement of interaction between the anchored chromophores within the polymer beads clearly contributes more.

In summary, we have shown that the solid phase work gives quite different reaction products when compared to analogous homogeneous conditions: a) it facilitates obtention of diesters against monoesters, b) the hydrolysis of the benzylic esters by MeO^- against HO^- is more selective under heterogeneous conditions, and c) in the DDQ/TFA treatment, again the solid phase work shows a higher selectivity in favour of condensation (i.e., tetrapyrrole formation) against oxidation (i.e., formyl pyrromethenone formation).

These examples illustrate how modification by the support of the chemical reactivity of attached compounds is indeed a methodology which can and must be exploited for synthetic purposes and in the obtention of structural information relative to these compounds; however, extreme care must be exercised in the interpretation of the observed results, and more specifically, in all factors which can influence these results.

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EXPERIMENTAL

Melting points were determined on a Kofler-Reichert microhot stage apparatus. UV-Vis spectra were recorded on a Perkin Elmer Lambda 5 instrument using in all cases quartz cuvettes. The KBr discs (0.85-0.95 mm thick x 10 mm diameter) used for the solid phase UV-Vis measurements were prepared under vacuum from 300 mg of dry KBr and 0.1-0.3 mg of the pigment, using a Perkin Elmer Evacuable Die 036103. The disk was then taken into a 1 mm thick quartz cuvette filled with Argon and tightly capped (parafilm). Mechanical grinding of the polymer beads (ca. 100 mg) was carried out in a Roto Mill (Kipp & Zonen) using agate cylinders (40 mm high x 15 mm i.d.). After 1 h grinding at the highest speed, CH_2Cl_2 is added to the resulting powder, and filtration through a 0.45 μm Millipore filter gives ca. 1/3 of the starting material which passes the filter and apparently "dissolves" in CH_2Cl_2 but precipitates in MeOH. The product thus obtained has been used for UV-Vis spectroscopic measurements (see below). Alternatively, the functionalized polystyrenes (ca. 20 mg plus a few drops of CH_2Cl_2) can be manually grinded in an agate mortar. The resulting powder is used for UV-Vis purposes as such or after filtering a suspension of it in CH_2Cl_2 through paper or cotton. In the spectroscopic determinations upon the polymer-bound pigments, the mechanically or manually ground sample (ca. 6 mg) respectively as a "solution" or suspension in 25 ml of the chosen solvent, is used. In the photoisomerization experiments, several AD spectra are recorded after irradiating the problem cuvette for increasing 3 min periods with one of the following light sources: 1) a high pressure Hg lamp (Phillips HPK, 125 W), 2) a 40 W or 100 W tungsten lamp. ^{1}H-NMR spectra were determined on a Perkin Elmer R-12 A, a Hitachi Perkin Elmer R 24-B (60 MHz) or on a Varian XL-200 (200.06 MHz) instrument. ^{13}C-NMR were obtained at 50.31 MHz on a Varian XL-200 spectrometer. The pH measurements have been performed at RT on a previously calibrated RADIO-METER PHM-51 instrument. Preparative TLC has been performed on 200 x200 mm plates containing a 1 mm thin layer of SiO_2 (Merck 60 HF_{254}). Analytical HPLC was carried out on Waters Radial Pak silica or C18 columns (0.8x 10 cm; particle size: 10 μm) with a Waters double pump (1 ml/min) using a variable wavelength detector 5 FA 339 (detection at 400 nm).
Dimethylformamide (DMF) was anhydrous and prepared from solvent grade DMF by vacuum distillation under an argon flow from P_2O_5 and ninhydrine. Shortly before every use it was thoroughly purged with argon. Absolute tetrahydrofurane (THF) was prepared from peroxides-free, dry THF by fresh distillation from $LiAlH_4$. All other solvents were purified following standard procedures[34], and argon saturated shortly before use. Aqueous 0.04 N solutions of cesium carbonate were prepared from the commercial (Merck) pure compound. Aqueous 1% tetra-n-butylammonium hydroxide was prepared from the commercial (Aldrich) 40% solution diluted with argon saturated water. Methyl Iodide was distilled shortly before use. Dichlorodicyanoparabenzoquinone, DDQ (Fluka) had been previously cristallized from benzene (M.p. 214- 222°).

Synthesis of Chromophores and Resins:

In the manipulation of all pyrrole pigments of this work, both O_2 and light must be avoided. BR IX-α (1a) is commercial (Sigma) and has been used without further purification; BR IX-α-DME (1c)has been obtained from BR by methylation with CH_2N_2 according to Blanckaert[35]. BV IX-α (2a) reagent grade has been prepared according to McDonagh by DDQ oxidation of BR[36]; MBV XIII-α-DME (2f) has been synthesized according to Falk[14] by DDQ/TFA catalyzed self condensation of XBRME; the respective free acid 2d has been obtained according to ref.[37] by base treatment of the diester 2f followed by slight acidification. XBRME (3c) and the corresponding free acid XBR (3a) have been synthesized as described in the literature[15] ; the N'-CH_3 derivatives of 3 have been obtained in a similar way but using the respective N'-CH_3 pyrrole 6d in the condensation step. The last has not been reported previously, and we have prepared it as described below.

Ethyl 1,2,4-Trimethyl-5-ethoxycarbonyl-1H-pyrrole-3-propanoate (6b). KO^tBu (0.47 g; 4.4 mmol) are added at RT under argon atmosphere to a solution of ethyl 2,4-dimethyl-5-ethoxycarbonylpyrrole-3-propanoate (6a)[15] (1.07 g; 4 mmol) in DMSO (38 ml). The mixture is stirred 45 min, then CH_3I (0.65 g; 6 mmol) are added and the stirring is maintained one additional hour at 60°. After this period, water (100 ml) are poured onto the reaction crude. The mixture is let to cool to RT and extracted with ether (3x 50 ml). The organic phase is washed with a NaCl saturated aqueous solution, dried with K_2CO_3, filtered and vacuum evaporated to dryness. The product is purified by SiO_2 column chromatography eluting with $CHCl_3$:MeOH (100:1); this gives the title pyrrole (0.97 g; 3.5 mmol; 86% yield) as an oil.
TLC (SiO_2; $CHCl_3$: MeOH; 100:1): R_f= 0.54
^{1}H-NMR ($CDCl_3$, δ , ppm): 4.25 (q, 2H, $-CO_2\underline{CH_2}CH_3$), 4.1 (q, 2H, $-CO_2\underline{CH_2}CH_3$), 3.7 (s, 3H, N-$CH_3$), 2.7-2.3 (m, 4H, $-CH_2CH_2$), 2.2 (s, 3H, $-CH_3$), 2.12 (s, 3H, $-CH_3$), 1.32 (t, 6H, $-CH_2\underline{CH_3}$).
IR (film, cm^{-1}): 1730 (C=O propanoate), 1680 (C=O carboxypyrrole).

1,2,4-Trimethyl-1H-pyrrole-3-propanoic Acid (6d). To a solution of 6b (295 mg; 1.05 mmol) in aqueous NaOH (208 mg; 5.2 mmol in 1 ml H_2O) enough ethanol (ca. 2 ml) is added dropwise, at RT, to dissolve all reaction mixture. The reaction flask is then heated in a steam bath and let to reflux during 5 h. The colour changes from yellow to orange. The ethanol is evaporated under vacuum and the warm residue (ca. 50°) under magnetic stirring is acidified with 10% aqueous HNO_3 (3.3 ml). The intermediate diacid (6c) thus obtained is decarboxylated without prior isolation. The flask containing the acidified mixture is equiped with an efficient Liebig condenser and heated for 30 min in the steam bath. The mixture is allowed to cool to RT, then extracted with ether (10x 15 ml), dried (Na_2SO_4), filtered and vacuum evaporated to dryness. This affords 1,2,4-trimethylpyrrole-3-propanoic acid (6d) (91 mg; 0.5 mmol; 48% yield) as an oil.
TLC (SiO_2; $CHCl_3$: MeOH; 100:1): R_f= 0.33

^{1}H-NMR ($CDCl_3$, δ , ppm): 6.2 (s, 1H, Ring-H), 3.3 (s, 3H, N-CH_3), 2.7-2.3 (m, 4H, -CH_2-CH_2-), 2.08 (s, 3H, -CH_3), 1.98 (s, 3H, -CH_3).
IR (KBr, cm^{-1}): 3300-2500 (COOH), 1710 (C=O), 1450, 1430, 1320, 1300, 1200 .

Methyl 5-[(2,5-Dihydro-3-ethyl-4-methyl-5-oxo-1H-pyrrol-2-ylidene)methyl]-1,2,4-trimethyl-1H-pyrrole-3-propanoate, XBRME-NMe (4c). To 1,2,4-trimethyl-1H-pyrrole-3-propanoic acid (227.9 mg; 2.91 mmol) is added a solution of (Z)-5-bromomethyliden-4-ethyl-3-methyl-3-pyrrolin-2-one[15] (629.4 mg; 2.91 mmol) in absolute MeOH (13.3 ml). The reaction mixture is refluxed 90 min in a water bath under argon and then ice-salt cooled to -5°. The solvent is evaporated under vacuum, and the solid residue is taken into a vacuum dessicator containing KOH. The dry residue dissolved in $CHCl_3$ (80 ml) is extracted with argon saturated aqueous 10 mM NaOH (6x 90 ml),then with water to neutral pH (3x 50 ml), dried with Na_2SO_4, filtered and evaporated to dryness. Purification by SiO_2 flash column chromathography eluting with $CHCl_3$:MeOH (100:1) gives the title product (621 mg; 1.88 mmol; 65% yield) as a pale-yellow solid of M.p.= 150-154° .
TLC (SiO_2; $CHCl_3$: MeOH; 10:1): R_f= 0.69
^{1}H-NMR ($CDCl_3$, δ , ppm): 7.02 (broad s, 1H, NH), 5.9 (s, 1H, -CH=), 3.68 (s, 3H, -CH_3 ester), 3.38 (s, 3H, N-CH_3), 2.75-2.35 (m, 6H, -CH_2-CH_2-, -CH_2-CH_3), 2.17 (s, 3H, -CH_3), 1.98 (s, 3H, -CH_3), 1.92 (s, 3H, -CH_3), 1.19 (t, 3H, -CH_2CH_3).
^{1}H-NMR (d_6-DMSO, δ , ppm): 9.23 (broad s, 1H, NH), 6.03 (s, 1H, -CH=), 3.60 (s, 3H, -CH_3 ester), 3.35 (s, 3H, N-CH_3), 2.75-2.35 (m, 6H, -CH_2-CH_2-, -CH_2-CH_3), 2.12 (s, 3H, -CH_3), 1.9 (s, 3H, -CH_3), 1.8 (s, 3H, -CH_3), 1.13 (t, 3H, -CH_2-CH_3).
IR (KBr, cm^{-1}): 1735 (ester C=O), 1670 (lactam C=O).
UV-Vis; λ_{max}, nm (ε): ($CHCl_3$): 392.6 (12,500), 263.3 (13,400). (EtOH): 401.1 (14,200), 263.7 (14,600).

5-[(2,5-Dihydro-3-ethyl-4-methyl-5-oxo-1H-pyrrol-2-ylidene)methyl]-1,2,4-trimethyl-1H-pyrrole-3-propanoic Acid, XBR-NMe (4a). To XBRME-NMe (4c) (301 mg; 0.91 mmol) in a 50 ml flask equipped with a reflux condenser, aqueous 10% NaOH (30 ml) are added, and the system, under magnetic stirring, is kept under reflux during 4 h. The flask is then cooled in an ice-salt bath and the solution is neutralized with aqueous 10% HNO_3 (ca. 40 ml). The flask under Ar is let to stir in the dark during 15 min at low temperature, then filtered, the precipitate washed with cold water and dried in a vacuum dessicator containing $CaSO_4$. This affords the title acid (250 mg; 0.79 mmol; 87% yield) as a yellow solid of M.p.= 190-195° .
TLC (SiO_2; $CHCl_3$: MeOH; 10:1): R_f= 0.25
^{1}H-NMR ($CDCl_3$, δ , ppm): 8.99 (broad s, 1H, NH), 6.0 (s, 1H, =CH-), 3.35 (s, 3H, N-CH_3), 2.74-2.50 (m, 6H, -CH_2-CH_2-, -CH_2-CH_3), 2.14 (s, 3H, -CH_3), 1.95 (s, 3H, -CH_3), 1.89 (s, 3H, -CH_3), 1.17 (t, 3H, -CH_2-CH_3).
^{1}H-NMR (d_6-DMSO, δ , ppm): 12.04 (s, 1H, -COOH), 9.62 (s, 1H, NH), 5.97 (s, 1H, =CH-), 3.35 (s, 3H, N-CH_3), 2.86-2.38 (m, 6H, -CH_2-CH_2-, -CH_2-CH_3), 1.87 (s, 3H, -CH_3), 1.80 (s, 3H, -CH_3), 1.76 (s, 3H, -CH_3), 1.13 (t, 3H, -CH_2-CH_3).
IR (KBr, cm^{-1}): 3300-2500 (-COOH), 1700 (C=O acid), 1645 (C=O lactam).
UV-Vis ($CHCl_3$); λ_{max}, nm (ε): 394 (12,900), 262 (11,400), 236 (9,000).

General Procedure for the Preparation of Bile Pigment Cesium Salts: The typical experimental conditions of this preparation are described here for BR IX-α.
A suspension of BR IX-α (1a) (58.4 mg; 0.1 mmol; 0.2 meq) in DMF (25 ml) is carefully neutralized at RT (pH meter is used; final pH= 9.5-10.0) with an aqueous 0.04 M Cs_2CO_3 solution (2.5 ml; 0.1 mmol; 0.2 meq). The solvent is removed below 30° under vacuum (rotary evaporator), then ca. 50 ml of dry benzene are added to the flask and vacuum evaporated to dryness; this procedure is repeated twice and the flask is finally kept overnight under vacuum in a dessicator containing P_2O_5. This yields dicesium bilirubinate, $BRCs_2$ (1b) (90 mg; 5 mg excess over the maximum expected) as a reddish orange solid which, by reverse phase HPLC (see below) is shown to consist of a mixture ca. 10:80:10 of the dicesium salts of respectively BR III, IX and XIII-α.
HPLC (C18; CH_3CN: 0.1 M aq. $AcONH_4$, 1:1): R_t (min): 6.2 (XIII-α), 7.9 (IX-α), 10.3 (III-α).
^{1}H-NMR (d_6-DMSO, δ , ppm): 12.7 (broad s, 1H, NH pyrrole), 12.5 (broad s, 1H, NH pyrrole), 12.2 (broad s, 2H, lactam NH), 7.8-5.2 (m, 6H, vinyls), 6.0 (s, 2H, =$C^{5,15}$H-), 3.8 (s, 2H, -$C^{10}H_2$-), 2.7-2.1 (m, 8H, propionate -CH_2-CH_2-), 2.12 (s, 3H, -$C^{17}H_3$), 2.06 (s, 3H, -$C^{13}H_3$), 2.02 (s, 3H, -C^7H_3), 1.86 (s, 3H, -C^2H_3).
IR (KBr, cm^{-1}): 3450, 3250, 1670, 1630, 1570, 1460, 1390, 1250.
UV-Vis (DMF); λ_{max}, nm (ε): 460 (ca. 35,000).

Biliverdin IX-α Dicesium Salt (2b).
^{1}H-NMR (d_6-DMSO, δ , ppm): 7.2 (s, 1H, =C^{10}H-), 6.2 (s, 2H, =$C^{5,15}$H-), 7.0- 5.7 (m, 6H, vinyls), 2.6-2.4 (m, 8H, -CH_2-CH_2-), 2.2 (s, 3H, -$C^{17}H_3$), 2.1 (s, 3H, -$C^{13}H_3$), 2.05 (s, 3H, -C^7H_3), 1.8 (s, 3H, -C^2H_3).
UV-Vis (DMF); λ_{max}, nm (ε): 381 (ca. 50,000), 730 (ca. 14,000).

Mesobiliverdin XIII-α Dicesium Salt (2e).
^{1}H-NMR (d_6-DMSO, δ, ppm): 7.2 (s, 1H, =C^{10}H-), 6.23 (s, 2H, =$C^{5,15}$H-), 3.3-2.4 (m, 12H, -CH_2-CH_2-, -CH_2-CH_3), 2.0 (s, 6H, -$C^{7,13}H_3$), 1.67 (s, 6H, -$C^{2,18}H_3$), 1.09 (t, 6H, -CH_2-CH_3).
UV-Vis (MeOH); λ_{max}, nm (ε): 366.4 (52,600), 635.5 (14,900).

XBR Cesium Salt (3b).
^{1}H-NMR (d_6-DMSO, δ , ppm): 11.03 (broad s, 1H, lactam NH), 10.44 (broad s, 1H, pyrrole NH), 5.87 (s, 1H, =CH-), 2.50-2.39 (m, 4H, one -CH_2- in -CH_2-CH_2-, -CH_2-CH_3), 2.09 (s, 3H, -C^3 or $^5H_3$), 1.95 (s, 3H, -C^5 or $^3H_3$), 1.95-1.89 (m, 2H, one -CH_2- in -CH_2-CH_2-), 1.74 (s, 3H, -$C^{3'}H_3$), 1.06 (t, J=6 Hz, 3H, -CH_2-CH_3).
IR (KBr, cm^{-1}): 3600-3200 (NH), 1670 (lactam), 1625 , 1570 (ester).

UV-Vis; λ_{max}, nm (ε): (EtOH): 418 (ca. 35,000). ($CHCl_3$): 408 (ca. 32,000).

XBR-NMe Cesium Salt (4b).
^{1}H-NMR (d_6-DMSO, δ , ppm): 9.66 (broad s, 1H, NH), 6.03 (s, 1H, =CH-), 3.35 (s, 3H, N-CH_3), 2.67-2.40 (m, 6H, $-CH_2-CH_2-$, $-\underline{CH_2}-CH_3$), 1.86 (s, 3H, $-CH_3$), 1.79 (s, 3H, $-CH_3$), 1.76 (s, 3H, $-CH_3$), 1.11 (t, 3H, $-CH_2-\underline{CH_3}$).
IR (film, cm^{-1}): 1690 (lactam C=O), 1570 (COO^-), 1390 (COO^-).
UV-Vis ($CHCl_3$); λ_{max}, nm (log ε): 409.4 (1.42), 262.4 (1.25), 203.1 (1.43).

Two different bromomethyl-Nbb resins (7a) with functionalities of approx. 1.05 and 0.65 meq Br per gram of resin have been obtained from commercial (Bio-Rad, 200-400 mesh beads) poly(styrene-co-1%-divinylbenzene) following the procedure reported in the literature[25].
The resin α-[(4-bromomethylbenzamido)benzyl]-poly(styrene-co-divinylbenzene) (so-called bromomethyldesnitro-Nbb), 7b, with a functionality of 0.9 meq per g of polymer has been prepared identically to 7a (see above; in the last step, the synthesis involves reaction of the 4-bromomethyl-3-nitrobenzoic anhydride with the amino groups of the so-called BHA resin), although omitting the nitration of the intermediate 4-bromomethylbenzoic acid. Two experimental differences are worth mentioning: the 4-bromomethylbenzoic anhydride is much less soluble in CH_2Cl_2 than its 3-nitro derivative; therefore much more CH_2Cl_2 is needed (260 ml for ca. 6 g of acid and ca. 6 g of dicyclohexylcarbodiimide, DCCI) in the preparation of 7b. Furthermore, in this case the intermediate anhydride must be let to react longer (24 h at RT) in order to achieve complete reaction with the amino groups of the resin. The IR spectrum of 7b is identical to that of 7a but lacks the bands of nitro group near 1540-1510 and 1350 cm^{-1}. Monomeric bromomethyl-Nbb (7c) can be synthesized in a similar way from benzophenone[38] .
The extension of all reactions involving resins has been monitored by IR spectroscopy, other specific assays, and by the weight gain of the functionalized resin. In the pigment binding processes, it is further confirmed by the amount of pigment recovered after base treatment of a given amount of linked chromophore.

General Procedure for the Attachment of Bile Pigment Salts to the Polymers 7a and 7b: The optimized experimental conditions of this attachment are described below for the anchoring of BR IX-α dicesium salt in DMF to the resin 7a with a functionality of 1.05. BR IX-α has also been attached to 7a through its di(tetra-n-butylammonium) salt in an identical procedure although using CH_2Cl_2 as the solvent.
To a given amount of beads of the bromomethyl-Nbb resin 7a (F= 1.05 meq Br/g resin; 302 mg; 0.317 meq) previously weighed in a 10 ml polypropylene syringe equipped with a perfectly fitted disk of polyethylene in its lower extreme (acting as a filter), is added $BRGs_2$ (1b) (the amount resulting from 97 mg; 0.17 mmol; 0.34 meq of BR and following the procedure described above) as a suspension in DMF (4x 1 ml are used to best wash the salt from the flask). The syringe is filled with argon, sealed with a "septum" and lightly Vortex-mixed in the dark, at RT during 24 h. The solvent is then filtered and the resin thoroughly washed with the following solvents (5 mlx 3 min each, with vigorous Vortex-mixing): 1) 2x DMF, 2) 2x 10% AcOH/CH_2Cl_2, 3) 1x CH_2Cl_2, 4) 1x DMSO, 5) 4x CH_2Cl_2, 6) 6x MeOH. The washed beads look deep garnet (almost black) and are dried overnight to constant weight in a vacuum dessicator containing P_2O_5. This gives diattached bilirubin, $BRCH_2Nbb$ (348 mg; 70% yield; final functionality in rubin is 0.58 meq BR/ g of resin).
The structure of this product and of all polymer-linked pigments that follow are further confirmed by alkaline MeOH detachment (see below) which gives the transesterification methyl esters.
IR (KBr, cm^{-1}): 3350, 3080-3020, 2920, 1745 (ester C=O), 1665 (resin amide C=O), 1635 (C=C), 1540 and 1350, (NO_2), 1280, 1150, 700.
UV-Vis; λ_{max}, nm (CH_2Cl_2, sample manually grinded); flat, broad band with maxima near 420, 460. ($CHCl_3$+ 1% EtOH, mechanically grinded): 395, 435 (sh). (DMSO, mechanically grinded): 385, 425 (sh). (KBr pellet): 420, 480 (sh).

Biliverdin-IX-α Bound to the Polystyrene 7a.
IR (KBr, cm^{-1}): 3480- 3280, 3080- 3020, 2920, 1740 (ester C=O), 1700, 1680-1640, 1600, 1540-1510, 1490, 1450, 1275, 1150, 700.
UV-Vis; λ_{max}, nm (CH_2Cl_2, sample manually grinded); 400, 720 ($\varepsilon_{400}/\varepsilon_{720}$ =ca. 1). ($CHCl_3$+ 1% EtOH, mecanically grinded): 378, 618 (very flat, it extends from 600 to 660).

Mesobiliverdin-XIII-α Bound to the Polystyrene 7a.
IR (KBr, cm^{-1}): 3400-3350 (NH), 1730 (ester C=O), 1690 (lactam C=O), 1670 (amide C=O), 1530 and 1350 (NO_2).

Xanthobilirubinic Acid Bound to the Polystyrene 7a.
IR (KBr, cm^{-1}): 3350 (NH), 1745 (ester C=O), 1680 (lactam C=O), 1670 (amide C=O), 1635 (C=C), 1550 and 1350 (NO_2), 1605, 1495, 1450, 760, 700 (polystyrene).
UV-Vis; λ_{max}, nm ($CHCl_3$+ 1% EtOH, sample manually grinded); 400. (EtOH, sample manually grinded): 405.
^{13}C-NMR ($CDCl_3$+ 10% MeOH, gel phase, δ , ppm)[11]: 172 (lactam and ester C=O), 145, 127 (resin), 117 (C^8, B-pyrrole), 116 (C^7, B-pyrrole), 98 (=C^5H-), 61 ($-COO\underline{C}H_2-$), 56, 39 (resin), 33 ($-\underline{C}H_2-COO-$), 28 (unreacted $-CH_2Br$), 19 ($-\underline{C}H_2-CH_2-COO-$), 17 ($-CH_3$ on lactam ring), 14 ($-\underline{C}H_2-CH_3$), 10 ($-CH_2-\underline{C}H_3$), 10, 7 (2x $-CH_3$ on pyrrole ring).

Xanthobilirubinic Acid N'-CH_3 Bound to the Polystyrene 7a.
IR (KBr, cm^{-1}): 3350 (NH), 1740 (ester C=O), 1680 (lactam C=O), 1535 and 1350 (NO_2).
UV-Vis (CH_2Cl_2); λ_{max}, nm: 385.
^{13}C-NMR ($CDCl_3$+ 10% MeOH, gel phase, δ , ppm)[11]: 172 (lactam and ester C=O), 145, 127 (resin), 117 (C^8, B-pyrrole), 116 (C^7, B-pyrrole), 98 (=C^5H-), 61 ($-COO\underline{C}H_2-$), 56, 39 (resin), 33 ($-\underline{C}H_2-COO-$), 30 (N'-CH_3), 28 (unreacted $-CH_2Br$), 19 ($-\underline{C}H_2-CH_2-COO-$), 17 ($-CH_3$ on lactam ring), 14 ($-\underline{C}H_2-CH_3$),

10 ($-CH_2-\underline{CH_3}$), 10, 7 (2x $-CH_3$ on pyrrole ring).

Xanthobilirubinic Acid Bound to the Polystyrene 7b.
IR (KBr, cm^{-1}): 3340 (NH), 1735 (ester C=O), 1680-1660 (lactam, amide C=O), 1630 (C=C).
UV-Vis; λ_{max}, nm ($CHCl_3$+ 1% EtOH); 400. (MeOH): 410.

Procedure for the Attachment of XBR Cesium Salt to the Monomer 7c. A mixture of xanthobilirubinic acid cesium salt (144 mg; 0.33 meq, prepared as described above), and the monomer 7c (141 mg; 0.33 meq) in DMF (20 ml) is made to react under magnetic stirring, at RT, in the dark, in a 100 ml flask under argon. The reaction is monitored every 30 min by TLC (SiO_2; $CHCl_3$: MeOH; 10: 1) of aliquots as follows: approximately 0.5 ml aliquots are taken, the solvent is evaporated at RT under vacuum and the organic part of the solid residue is dissolved in $CHCl_3$ containing ca. 2% MeOH (XBR bound to 7c is too insoluble in pure $CHCl_3$). The resulting ester has similar Rf to that of XBRME (3c)(see below), while the more polar starting salt migrates less. After two hours the reaction is complete. The DMF is vacuum evaporated below 30°, the solid residue is thoroughly washed with H_2O (4x 20 ml) to dissolve the CsBr and the residue is dried to constant weight in a vacuum dessicator (P_2O_5). This leaves the title ester (192 mg; 90% yield) as a yellow solid very insoluble in most solvents assayed.
M.p.= 214-229° (most of it between 226-229°; byproduct might be some E-isomer).
TLC (SiO_2; $CHCl_3$: MeOH; 10: 1): Rf= 0.72
^{1}H-NMR (d_6-DMSO, δ , ppm): 10.31 (s, 1H, pyrrole NH), 9.76 (s, 1H, lactam NH), 9.63 (d, 1H, amide NH), 8.60 (s, 1H, ortho-H^{ar}), 8.26 (d, 1H, para-H^{ar}), 7.68 (d, 1H, meta-H^{ar}), 7.35 (broad band, phenyl), 6.40 (d, 1H, $-CONH-\underline{CH}-$), 5.91 (s, 1H, $=\underline{CH}-$), 5.46 (s, 2H, $-COO-\underline{CH_2}-$), 2.80-2.30 (m, 6H, $-\underline{CH_2}-CH_3$, $-(\underline{CH_2})_2-COO-$), 2.15 (s, 3H, $-CH_3$), 2.02 (s, 3H, $-CH_3$), 1.76 (s, 3H, $-CH_3$), 1.06 (t, 3H, $-CH_2-\underline{CH_3}$).
^{1}H-NMR ($CDCl_3$:CD_3OD; 5:1, δ , ppm): 8.60 (s, 1H, ortho-H^{ar}), 8.16 (d, 1H, para-H^{ar}), 7.58 (d, 1H, meta-H^{ar}), 7.35 (broad band, phenyl), 6.47 (d, 1H, $-CONH-\underline{CH}-$), 6.09 (s, 1H, $=\underline{CH}-$), 5.53 (s, 2H, $-COO-\underline{CH_2}-$), 2.75 (q, 2H, $-\underline{CH_2}-CH_3$), 2.56 (m, 4H, $-(\underline{CH_2})_2-COO-$), 2.26 (s, 3H, $-CH_3$), 2.13 (s, 3H, $-CH_3$), 1.90 (s, 3H, $-CH_3$), 1.16 (t, 3H, $-CH_2-\underline{CH_3}$).

IR (KBr, cm^{-1}): 3340 (NH), 1735 (ester C=O), 1660 (lactam C=O), 1640-1630 (amide C=O, C=C), 1525 and 1335 (NO_2), 695.
UV-Vis; λ_{max}, nm (ε) (EtOH): 411 (25,000). (Dry $CHCl_3$): 399 .

Reaction of $BRCs_2$ (1b) and BR $(NBu_4)_2$ (1d) with CH_3I. The method given here corresponds to the reaction of $BRCs_2$ with CH_3I in DMF. For $BR(NBu_4)_2$ everything is identical but CH_2Cl_2 is the solvent. The composition of the reaction mixture in the last case is given in Table 2.
To a solution of $BRCs_2$ (1b) (17 mg; 0.02 mmol; 0.04 meq) in DMF (6 ml) contained in a 25 ml flask, CH_3I (4 drops, ca. 200-400 mg; ca 0.2 meq, 10-fold excess) is added at once. The flask is tightly capped and magnetically stirred at RT in the dark during 10 min. The solution is then evaporated at RT under vacuum (ca. 1 mm) and the resulting solid dissolved in $CHCl_3$ (25 ml), washed with 3% (4x 25 ml) aqueous HCl to neutralize any remaining carboxylate, then with H_2O to neutral pH, dried (Na_2SO_4), filtered, evaporated to dryness and used for spectroscopic identification. TLC (SiO_2; $CHCl_3$: MeOH: AcOH; 100: 2: 1) comparing to standards of BR (1a) and BRDME (1c) shows two spots with same Rf as the standards, plus a third one with intermediate Rf (BR monoester).
HPLC (C18; CH_3CN: 0.1 M aqueous $AcONH_4$; 1:1 for 18.50 min, then 15 min gradient up to 85% CH_3CN): R_t, min: 5.25, 7.05, 9.51 (scrambled mixture of BR isomers; ca. 19%), 10.98, 12.11, 13.51 (scrambled mixture of BRMME isomers: ca. 54%), 35.65 (BRDME; ca. 27%).
UV-Vis (CH_2Cl_2); λ_{max}, nm: 400 (BRDME), 434 (BRMME) (relative intensities, ca. 1: 2). BR is insoluble in this solvent.
IR (KBr, cm^{-1}): 1740 (ester C=O), 1700 (free acid C=O), 1660 (lactam C=O), 1630 (C=C).
The ^{1}H-NMR in $CDCl_3$ shows the NH signals of BRDME[19b] at 11.23- 10.10 and of BRMME[39] at 10.83- 10.73, 9.29- 9.17 and 8.60- 8.41. BR is insoluble in this solvent.

Reaction of $BRCs_2$ (1b) and BR $(NBu_4)_2$ (1d) with the Monomer 7c. The method given here corresponds to the reaction of $BRCs_2$ with the monomer 7c in DMF. For $BR(NBu_4)_2$ everything is identical but CH_2Cl_2 is the solvent. The reaction mixture in the last case is given in Table 2.
To a solution of $BRCs_2$ (1b) (17 mg; 0.02 mmol; 0.04 meq) in DMF (6 ml) contained in a 25 ml flask, the monomer 7c (17 mg; 0.04 meq) is added at once and the capped flask is magnetically stirred at RT in the dark during 10 min. The work-up as described for the reaction with CH_3I gives a greenish yellow solid used for spectroscopic identification. TLC (SiO_2; $CHCl_3$: MeOH: AcOH; 100: 2: 1) comparing to BR (1a), BRDME (1c) and the monomer 7c shows three spots with same Rf as the standards, BR and 7c being by large the most important ones, plus a fourth spot with intermediate Rf (BR monoester). The BR esters of 7c are too insoluble in all solvents assayed to permit their HPLC analysis.
UV-Vis (CH_2Cl_2); λ_{max}, nm: 399 (BR diester), 440 (BR monoester) (relative intensities, ca. 1: 1). BR is insoluble in this solvent.
IR (KBr, cm^{-1}): 1740 (small, ester C=O), 1700 (large, free acid C=O), 1660 (lactam C=O), 1630 (C=C), also bands of unreacted 7c[38].
The ^{1}H-NMR in $CDCl_3$: CD_3OD; 1:1 shows the signals of BR-$COOCH_2$- as a singlet near 5.36 ppm and of unreacted Br-CH_2- near 4.76 ppm. with relative integration 30: 70% .

General Procedure for the Detachment of Bile Pigments from their Esters with the Polystyrenes 7a and 7b. The optimized experimental conditions of this are described below for the hydrolysis of BR IX-α bound to 7a. Detachment of biliverdins is simpler since neither disproportionation nor monoesterification occurs.
Polystyrene anchored BR (approximate functionality in BR= 0.58 meq BR/g; 50 mg; ca. 0.029 meq BR, ca. 9 mg BRDME expected) are weighed in a polypropylene syringe equipped as the one used in the

attachment process. At RT and in the dark, the coloured beads are washed (3x 3 mlx 2 min) under vigorous Vortex mixing with an argon saturated, recently prepared mixture of dioxane:MeOH:4N NaOH (30:9:1) and the solvent after each washing filtered onto a mixture of $CHCl_3$ (9 ml) and an aqueous glycine/HCl buffer (4.5 ml, starting pH= 2.7, final pH= 5-6). The first filtrate is deep orange, while the third is nearly colourless. Alternatively, after each base washing, another with pure MeOH can be performed to better remove all methyl ester formed. The remaining resin has a lighter garnet colour. The solvent mixture is taken into a separatory funnel, and the aqueous phase is washed with $CHCl_3$ (4x 20 ml) which takes all colour, filtered through paper and evaporated. This yields 8 mg (90% of the maximum) of a completely scrambled mixture of BR III-, IX-, and XIII-α dimethyl esters (by reverse-phase HPLC), containing ca. 10% of BR monoester (by ^{1}H-NMR).
TLC(SiO_2; $CHCl_3$: MeOH: AcOH; 97: 2: 1) comparing to BR (R_f= 0.64) and to BRDME prepared independently (R_f= 0.24) shows, most important, BRDME and only traces of a third species with R_f= 0.29 (BRMME?).
All other analytical data (HPLC, C18; IR; ^{1}H-NMR) confirm a mixture of isomerized BRDME (ca. 90%) and BRMME[19b,39].

Detachment of XBR from its Ester with the Monomer 7c. The ortho-nitrobenzylic ester of XBR and 7c prepared as indicated above (25 mg; 0.039 meq) in a 50 ml flask is treated at RT, under vigorous stirring, with a mixture of Dioxane:MeOH:4N NaOH (30: 9: 1) (10 ml) during 5 min. An aqueous glycine/HCl buffer solution (pH= 2.7) is added until a pH close to 5.5 is reached (a slight colour change is observed; ca. 1.5 ml are needed). The resulting solution is poured onto a mixture of $CHCl_3$: H_2O (25+ 25 ml) in a separatory funnel, and the organic phase is separated and worked up as usual. This gives a yellow greenish mixture determined by TLC (SiO_2; $CHCl_3$:MeOH; 10:1) to consist of XBRME (R_f= 0.68), XBR (R_f= 0.33), and the monomer HO-CH_2-Nbb (R_f= 0.49; -OH band in the IR). Preparative TLC under the same conditions leaves XBRME (9.5 mg; 0.030 meq; 76% yield), XBR (2.5 mg; 0.008 meq; 21%), and HO-CH_2-Nbb (9.8 mg; 0.027 meq; 67%).
IR (KBr, cm^{-1}) of HO-CH_2-Nbb: 3600-3400 (OH), 3295 (NH), 1630 (amide C=O), 1530 and 1340 (NO_2).

Methyl 5-[(2,5-dihydro-3-ethyl-4-methyl-5-oxo-1H-pyrrol-2-ylidene)methyl]-2-formyl-4-methyl-1H-pyrrole-3-propanoate, XBRME-5'-CHO. The procedure given here corresponds to the conditions of Assay #3 in Table 5, which affords the highest yield of aldehyde.
XBRME (3c) (22 mg; 0.067 meq) in absolute THF (2 ml) under argon, are taken with magnetic stirring into an ice-salt bath. Onto the mixture is added at once and in this order, TFA (1 ml) and a solution of DDQ (31 mg; 0.14 meq) in absolute THF (3 ml). The colour changes from yellow to green. After 90 min stirring at low temperature, H_2O (1 ml) is added and the flask is let to stir at low temperature during 30 more minutes. A mixture of 10% aqueous Et_3N (3 ml), $CHCl_3$ (5 ml) and ascorbic acid (traces) is then added, and the organic phase is separated from the mixture in a separatory funnel. The aqueous phase is washed with $CHCl_3$ (2x 3 ml), and the combined organic extracts are washed first with 10% aqueous Et_3N (4x 4 ml) until no yellow colour is taken, then with 0.4 M aqueous HCl (2x 4 ml), H_2O to neutral pH, dried (Na_2SO_4), filtered and evaporated under vacuum. TLC (SiO_2; $CHCl_3$:MeOH; 10:1) shows two spots corresponding to MBV XIII-α-DME (2f)[14a] (blue), and the title aldehyde[40] (yellow). Preparative TLC under the same conditions affords 4.9 mg of verdin (0.008 mmol; 23%) and 12.9 mg of aldehyde (0.037 mmol; 54%), as a solid of M.p.= 211° (Lit.[40]: 211°)
IR (film, cm^{-1}): 3340 (NH), 1735 (ester C=O), 1690 (aldehyde C=O), 1670 (lactam C=O), 1630 (C=C).
^{1}H-NMR ($CDCl_3$, δ, ppm): 10.7 (broad s, 1H, lactam NH), 10.3 (broad s, 1H, pyrrole NH), 9.8 (s, 1H, -CHO), 5.95 (s, 1H, =CH-), 3.65 (s, 3H, $-COOCH_3$), 3.0-2.5 (m, 6H, $-CH_2-CH_2-$, $-CH_2-CH_3$), 2.1 (s, 3H, $-CH_3$), 1.95 (s, 3H, $-CH_3$), 1.2 (t, 3H, $-CH_2-CH_3$).
UV-Vis (MeOH); λ_{max},nm (ε): 394 (27,200), 415 (23,100).

DDQ/TFA Catalyzed Self Condensation of XBR Bound to Polystyrene 7a. TFA (1 ml) is added at once to a sample of XBR bound to functionalized polystyrene 7a (approximate functionality in XBR= 0.88 meq XBR/g, 100 mg; 0.088 meq) in absolute THF (2 ml) contained in a 10 ml polypropylene syringe identical to that described in previous headings. The syringe is filled with argon, tightly capped with a septum and kept in a cold room at 5 under light Vortex mixing. After ca. 15 min, with the help of another syringe equipped with a needle, a solution of DDQ (40 mg; 0.18 meq) in absolute THF (3 ml) is added. The addition is completed in 30 min, the cold mixture is let to shake during 60 more minutes, then filtered and the dark resin washed with the following solvents (4 mlx 2 min under vigorous Vortex mixing each): 1) 3x CH_2Cl_2, 2) 4x 5% EDTA in CH_2Cl_2, 3) 3x THF, 4) 3x MeOH (or until neutral pH). The first washings are deep yellow, but the last colourless. The resin is dried overnight in a vacuum dessicator with P_2O_5, and the final weight is 99.3 mg. Its composition is determined by alkaline MeOH detachment (see above). From 50 mg of the product resin, 13.7 mg of a green product are obtained (quantitative detachment). TLC and ^{1}H-NMR show a mixture of XBRME-5'-CHO (5.9 mg; 0.018 meq; 45% yield), MBV XIII-α-DME (7.3 mg; 0.024 meq; 52%), MBR XIII-α-DME (ca. 5%), and XBRME (ca. 5%).

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