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Tetrahedron
Tetrahedron 60 (2004) 8669–8675

Gaining diversity in solid-phase synthesis by modulation of cleavage conditions from hydroxymethyl-based supports. Application to lamellarin synthesis

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Received 9 December 2003; accepted 4 May 2004

Available online 10 August 2004

Abstract—The application of a number of Lewis acids as a cleavage/deprotection method in the solid-phase synthesis of organic molecules can render several analogues, which, after purification, can be submitted for biological evaluation.

1. Introduction

Although combinatorial chemistry has evolved greatly since its early days in peptide chemistry, it is undoubtedly an essential tool in modern programs of medicinal chemistry.[1] Combinatorial chemistry is currently being redirected towards the rapid and rational preparation of small and medium sized libraries of compounds based on a determined scaffold.[2]

Library preparation can be carried out in solid-phase[3] or in solution, by mainly taking advantage of the solid-phase mode through the use of supported reagents and/or supported purification probes.[4] Library diversity is normally introduced during the incorporation of the building blocks. However, when the library is prepared in solid-phase, diversity can also be introduced during cleavage. Thus, there are resins specially designed for this purpose.[5] These resins usually release the final compounds by means of a nucleophile. The oxime resin developed by Kaiser and DeGrado[6] and the 'safety-catch' resins developed by Kenner[7] and Ellman,[8] and the aryl hydrazine linker of Lowe[9] are examples of it.[10] Another new concept introduced by combinatorial chemistry is that any side-product is valuable because after purification it can be submitted for biological screening.[11]

Here we describe an example of how the synthesis of the distinct analogues of a natural product can be obtained by using different cleavage conditions, and even by modulating these conditions.

For this study, lamellarins were chosen as substrates. These hexacyclic alkaloids were first isolated from *Lamellarina* sp in 1985 by Faulklner et al.[12] Some lamellarins exhibit a wide array of interesting and significant biological activities, which include cell division inhibition, cytotoxicity, HIV-1 integrasa inhibition and immunomodulation.[13] While the first synthesis of lamellarins was carried out in 1997, our group has recently reported the first solid-phase synthesis of these hexacyclic alkaloids (Fig. 1).[14]

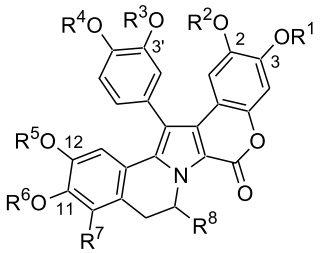

Figure 1. General formula of lamellarins.

2. Results and discussion

Some lamellarins have phenol functions at C3 and C3′ (R^1, R^3=H), therefore a plausible way to start solid-phase synthesis is by anchoring the phenol at C3′ to a solid support. Merrifield and Wang resins are convenient for this process because they shown a good mechanical and

Keywords: Combinatorial chemistry; Diversity; Libraries; Acidolytic cleavage.
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doi:10.1016/j.tet.2004.05.119

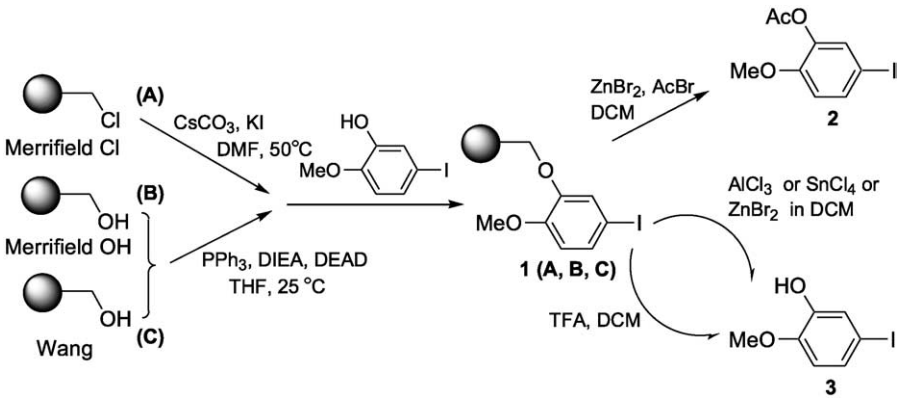

Figure 2. Incorporation and cleavage of the first building block.

chemical stability, highest loading and the lowest cost, which make these resins the most suitable solid supports for a multi-step solid-phase synthesis.[5] These resins, which are widely used in peptide synthesis for the preparation of acid peptides, release the final compounds after an acid treatment. While TFA (for the Wang resin), which is the method of choice for peptide cleavage, is a convenient reagent for combinatorial chemistry, its counterparts for Merrifield resin, anhydrous HF or TFMSA, do not appear to be appropriate. During recent years several methods based on the use of Lewis acids have been used to cleave small molecules from this type of resins.[15] Thus, here we have checked some of these methods in the synthesis of lamellarins to obtain different analogues. Resins were cleaved with a Lewis acid such as $AlCl_3$,[16] $SnCl_4$,[17] $ZnBr_2$[18] or with carboxylic acids such as TFA in DCM.

Three different polystyrene resins, chloro and hydroxy Merrifield and Wang resins were tested. 2-Methoxy-5-iodophenol was incorporated to Merrifield-Cl resin by the cesium salt method ($CsCO_3$, KI, in DMF at 50 °C for 24 h, the process was repeated once), and to the hydroxy resins via Mitsunobu conditions (PPh_3, DIEA, DEAD in THF at 25 °C for 3 h). Furthermore, Merrifield-Cl was also rejected because the incorporation of the phenol required more drastic conditions (double process at 50 °C for 24 h) and because the reaction cannot be followed by any colorimetric test or FT-IR.[19]

Initially, 2-methoxy-5-iodophenol was recovered with good yield and purity in all the cleavage cases, but in the $SnCl_4$ cleavage the purity of the compound was not acceptable. Thus, we discarded this reagent. When the $ZnBr_2$ cleavage is carried out into the presence of acetyl bromide the corresponding acetyl derivative is obtained with good yield and purity. Thus seems, the possibility of using another acyl bromide to increase the diversity (Fig. 2).

Lamellarin U (R^2, R^3=H) was taken as a model and its structure was built up in the two hydroxymethyl resins. The hydroxyl group of the OH in position 3 was protected as isopropyl ether, which can be cleaved by acids,[20] if it is

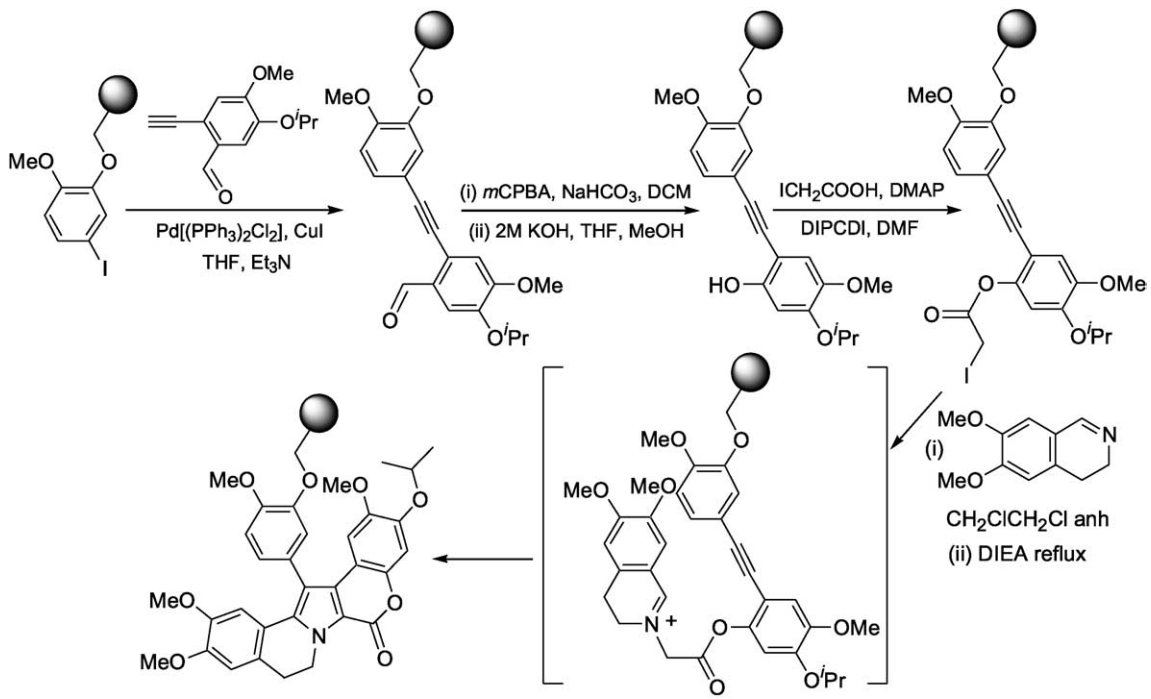

Figure 3. Solid-phase synthesis of lamellarins.

desired. Other MeO groups were already introduced into the building blocks. For the Wang resin, the scheme outlined in Figure 3 was followed, starting with a functionalization of 0.86 mmol/g. The process is similar, with minor modifications, to that followed for the solid-phase preparation using Merrifield-OH.[14]

Cleavages were performed with the reagents discussed above. As expected, cleavage with $ZnBr_2$ (4 equiv.) in dry DCM in the presence of AcBr (overnight, 10 equiv.) gave the acetyl derivatives corresponding to 3,3′-di-*O*-acetyllamellarin U. Cleavage with $AlCl_3$ in dry DCM (10 equiv.) produced mainly lamellarin U, accompanied by lamellarin L (free hydroxy at C11) and 12-*O*-demethoxylamellarin U (free hydroxy at C12). Although the main product was always Lamellarin U, the proportion of other derivatives can be increased by extending cleavage times (3–6 h). With a shorter cleavage time, the compound with an isopropoxy group at C3 was also obtained. This derivative (3-*O*-isopropyllamellarin U) was the main product when the cleavage was performed with TFA on Wang resin. In this cleavage, an unexpected derivative containing a chlorine atom at C2′ (2′-chloro-3-*O*-isopropyllamellarin U) was also obtained.[21] The purity of compounds obtained with the Merrifield resin is greater than when the Wang resin is used. Therefore, although cleavages carried out with Lewis acids can also be performed on Wang resin, the results obtained with Merrifield resins were better (Fig. 4).

In conclusion, the application of a number of Lewis acids as a cleavage/deprotection method in the solid-phase synthesis of organic molecules can be used as an important diversity point rendering several analogues, which, after purification, can be submitted for biological evaluation.

3. Experimental

3.1. General procedure

Hydroxymethyl poly(styrene-*co*-1% divinylbenzene) resin (Merrifield-OH, 100–200 mesh material, nominal loading: 0.68 mmol/g); chloromethyl poly(styrene-*co*-1% divinylbenzene)resin (Merrifield-Cl resin, 100–200 mesh material, nominal loading: 0.61 mmol/g), 4-hydroxymethylphenoxymethyl poly(styrene-*co*-1% divinylbenzene) resin (Wang resin, 100–200 mesh material, nominal loading: 0.86 mmol/g) were from NovaBiochem (Läufelfingen, Switzerland).

Tetrahydrofuran (THF) was freshly distilled from sodium/benzophenone. Dichloromethane was distilled from calcium hydride prior to use. DMF (99.99% anhydrous) was purchased from SDS and used as received.

^{1}H NMR and heterocorrelations (600 MHz) spectra were recorded on Bruker spectrometer, ^{13}C NMR (100 MHz) spectra were recorded on a Varian Mercury 400 spectrometer. Chemical shift (δ) are expressed in parts per million downfield from $CDCl_3$ as internal standard.

Analytical HPLC was carried out on a Waters® 2695

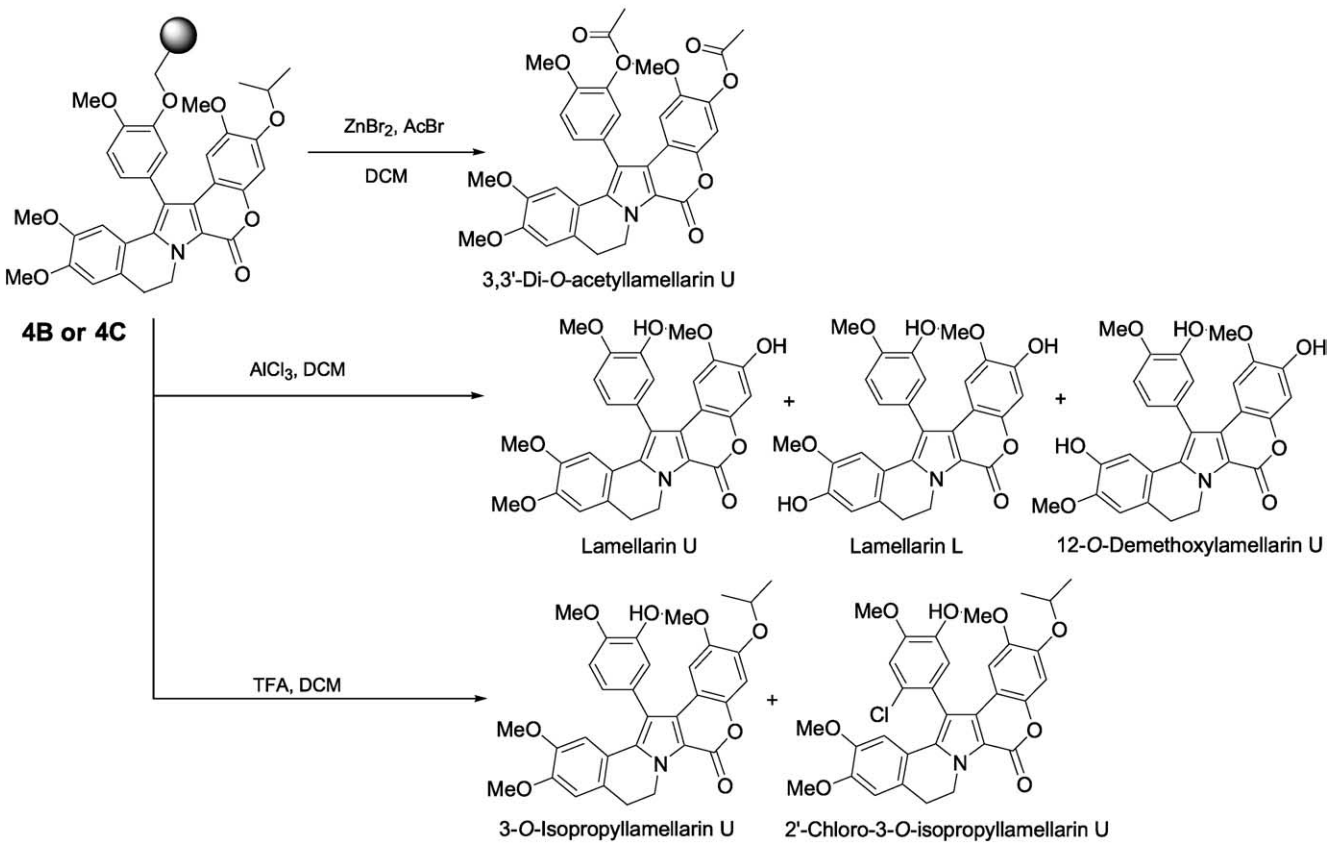

Figure 4. Lewis acid cleavage of solid-phase supported lamellarins.

Separations Module instrument, Water® 996 Photodiode Array Detector. UV detection from 210 to 500 nm and linear gradients of CH_3CN into H_2O were run at 1.0 ml/min flow rate from: 70:30 to 0:100 over 15 min, with a Symmetry® C-18 5.0 μm 4.6 mm×150 mm column. Preparative HPLC was carried out on a Waters® 600 Multisolvent Delivery System instrument, Waters® 2700 Sample Manager and a Water® 2487 Dual Absorbance UV Detector; detection at 277.0 and 315.0 nm. Linear gradients of CH_3CN into H_2O were run at 25.0 ml/min flow rate from: 70:30 to 40:60 over 60 min, with a Symmetry® C-8 5.0 μm 30 mm×100 mm column.

$APCI^+$-MS analysis of crude material and final compounds were performed in a Mass spectrometer VG Platform II, Micromass.

FAB^+ HR-MS were performed on a Autospec FAB+by Unidade de Espectrometría de Masas (Universidad de Santiago de Compostela).

3.2. Typical procedure for iodophenol anchorage to the hydroxy type resins (hydroxy Merrifield and Wang resins)

3.2.1. 5-Iodo-2-methoxyphenoxy-resin (1b, 1c). The procedure described by Spatola et al.[22] for the incorporation of Tyr trough a Mitsunobu reaction has been repeated here for the incorporation of 2-methoxy-5-iodophenol to Merrifield-OH and Wang resins. Merrifield-OH resin (1.0 g of 100–200 mesh material, 0.68 mmol/g loading) was washed with DCM (1×10 ml) and THF (1×10 ml). The dried resin was then treated with THF (15 ml), 2-methoxy-5-iodophenol (510 mg, 2.04 mmol, 3 equiv.), and triphenylphosphine (535 mg, 2.04 mmol, 3 equiv.), diisopropylethylamine (1.05 ml, 6.12 mmol, 9 equiv.) and the resulting mixture was stirred and cooled at 0 °C. Diethyl azodicarboxylate (320 μl, 2.04 mmol, 3 equiv.) was then added dropwise and the resulting mixture was stirred 3 h at room temperature. The solvent was then removed. The resin was washed with DMF, DCM, methanol and diethyl ether (5×15 ml each) and dried under reduced pressure. IR (KBr, cm^{-1}), in the IR spectrum it is possible to see the absence of at 3450 and 3580 cm^{-1} proper of hydroxyl groups of the resin; ^{13}C NMR-MAS (125 MHz) δ 149.8, 149.2, 130.1 (C-3), 122.7 (C-4), 113.7 (C-6), 82.2 (C-5), 55.9 (OCH_3).

3.2.2. 5-Iodo-2-methoxyphenoxy-resin (1a). Merrifield-Cl resin (1.0 g of 100–200 mesh material, 0.61 mmol/g loading) was washed with DCM (1×10 ml). The dried resin was then swelled in DMF (10 ml) for 30 min, then cesium 2-methoxy-5-iodo-phenolate (1.38 g, 3.6 mmol, 6 equiv.) was added in DMF (dry), finally KI (20 mg, 012 mmol, 0.2 eq.) was added. The resulting mixture was stirred at 50 °C for 24 h. It was then filtered and washed with DMF, DCM, MeOH and diethyl ether (5×15 ml each) and dried under reduced pressure. Spectroscopic data were similar as described in the previous example.

3.3. Typical procedure for the cleavage with anhydrous $SnCl_4$

3.3.1. 5-Iodo-2-methoxyphenol (3).[23] The resin **1A** or **1B** or **1C** (100 mg, 0.5 mmol/g theoretical loading) was swelled with dry DCM for 30 min, $SnCl_4$ (10 equiv.) was added and the reaction mixture was shaken under Ar overnight. After this time the resin was filtered off and washed with DCM (5×5 ml). The filtrates were washed with H_2O (6×15 ml), dried ($MgSO_4$ anhydrous), and concentrated under reduced pressure to give 5-iodo-2-methoxyphenol (12 mg of crude material, 70% of purity by HPLC). ^1H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, J=2.2 Hz, 1H, H-6), 7.15 (dd, J=8.4, 2.2 Hz, 1H, H-4), 6.59 (d, J=8.4 Hz, H-3), 5.60 (bs, 1H, OH), 3.87 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 146.6 (s), 146.5 (s), 129.0 (d, C-3), 123.4(d, C-4), 112.4 (d, C-6), 83.0 (s, C-5), 56.0 (q, OCH_3).

3.4. Typical procedure for the cleavage with anhydrous $ZnBr_2$

3.4.1. 5-Iodo-2-methoxyphenol (3). The resin **1A** or **1B** or **1C** (100 mg, 0.5 mmol/g theoretical loading) was swelled for 30 min with dry DCM 1 ml). After this time anhydrous $ZnBr_2$ (4 equiv.) were added and the reaction mixture was then shaken under Ar for 4 h at room temperature. The resin was filtered off and washed with DCM (5×5 ml). The filtrates were washed with aq. 5% $NaHCO_3$ (5×15 ml), 2 M HCl (3×15) and brine (3×15 ml). The organic solution was dried ($MgSO_4$ anhydrous), filtered and concentrated under reduced pressure to give 5-iodo-2-methoxyphenol (10.5 mg, 84%); CIMS m/z (relative intensity): 250 ([M^+],100). Spectroscopic data were the same as in the example above.

3.4.2. 5-Iodo-2-methoxyphenol acetate (2). The resin **1A** or **1B** or **1C** (100 mg, 0.5 mmol/g theoretical loading) was swelled for 30 min with dry DCM (1 ml). After this time anhydrous $ZnBr_2$ (4 equiv.) and AcBr (10 equiv.) were added and the reaction mixture was then shaken under Ar overnight at room temperature. The resin was filtered off and washed with DCM (5×5 ml). The filtrates were washed with aq. 5% $NaHCO_3$ (5×15 ml), 2 M HCl (3×15) and brine (3×15 ml). The organic solution was dried ($MgSO_4$ anhydrous), filtered and concentrated under reduced pressure to give 5-iodo-2-methoxyphenol acetate (13 mg, 89%). ^1H NMR (200 MHz, $CDCl_3$) δ 7.49 (dd, J=8.8, 2.0 Hz, 1H, H-4), 7.33 (d, J=2.0 Hz, 1H, H-6), 6.72 (d, J=8.8 Hz, H-3), 3.81 (bs, 3H, OCH_3), 2.30 (s, 3H, $COCH_3$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.5 (s, C=O), 151.3 (s, C-2), 140.4 (s, C-1), 135.6 (d, C-4), 131.5 (d, C-6), 114.2 (d, C-3), 81.3 (s, C-5), 56.0 (q, OCH_3), 20.6 (q, $COCH_3$).

3.4.3. 3,3′-Di-*O*-acetyllamellarin U. The resin **4B** (180 mg, 0.48 mmol/g theoretical loading) was swelled for 30 min with dry DCM (1 ml). After this time anhydrous $ZnBr_2$ (225 mg, 4 equiv.) and AcBr (111 mg, 10 equiv.) were added and the reaction mixture was then shaken under Ar overnight at 25 °C. The resin was filtered off and washed with DCM (5×5 ml). The filtrates were washed with aq. 5% $NaHCO_3$ (5×15 ml), 2 M HCl (3×15) and brine (3×15 ml). The organic solution was dried ($MgSO_4$ anhydrous), filtered and concentrated under reduced pressure. The crude was analyzed by HPLC-MS [C18-$APCI^+$ using H_2O (5 mM $AcNH_4$): acetonitrile gradient 30–100% acetonitrile in 15 min]: retention time 9.73 min, molecular weight 599.58; found 600.6 $[M+H]^+$. MSMS (Q-TOF) calcd 599.18; found 600.20 $[M+H]^+$, 557, 22

$[(M+H)^+-C_2H_2O]$, 515.29 $[(M+H)^+-2\times C_2H_2O]$. The crude product was purified by HPLC and the 3,3′-di-*O*-acetyllamellarin U (4 mg, 8.5% overall yield) was obtained. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30 (dd, J=8.2, 2 Hz, 1H, H-6′), 7.22 (d, J=2 Hz, 1H, H-2′), 7.12 (d, J=8.2 Hz, 1H, H-5′), 7.06 (s, 1H, H-4), 6.74 (s, 1H, H-10), 6.72 (s, 1H, H-1), 6.62 (s, 1H, H-13), 4.95 (m, 1H, H-8), 4.64 (m, 1H, H-8), 3.87 (s, 6H, C^{12}-OCH_3 and $C^{4'}$-OCH_3), 3.44 (s, 3H, C^2-OCH_3), 3.40 (s, 3H, C^{11}-OCH_3), 3.15 (m, 1H, H-9), 3.05 (m, 1H, H-9), 2.29 (s, 3H, C^3OOCCH_3), 2.27 (s, 3H, $C^{3'}$OOCCH_3); ^{13}C NMR (150 MHz, $CDCl_3$)[24] δ 168.8 (C^3OO*C*CH_3), 168.3 ($C^{3'}$OO*C*CH_3), 151.2 (C-4′), 149.0 (C-12), 147.5 (C-11), 147.4 (C-2), 144.8 (C-3), 140.7 (C-3′), 138.7 (C-4a), 136.1 (C-13b), 129.5 (C-6′), 127.7 (C-1′), 127.4 (C-14a), 126.4 (C-9a), 125.0 (C-2′), 119.8 (C-13a), 116.1 (C-14b), 114.1 (C-14), 112.5 (C-5′), 111.8 (C-4), 110.9 (C-10), 108.5 (C-13), 105.4 (C-1), 56.0 ($C^{4'}$-O*C*H_3), 56.0 (C^{12}-O*C*H_3), 55.6 (C^2-O*C*H_3), 55.2 (C^{11}-O*C*H_3), 42.5 (C-8), 28.7 (C-9), 20.0 ($C^{3'}$OOC*C*H_3), 20.0 (C^3OOC*C*H_3). (+)-HRMS *m*/*z* 599.1789 (calcd for $C_{33}H_{29}NO_{10}$ $[M]^+$ 599.1791, Δ +0.4 ppm).

3.5. Typical procedure for $AlCl_3$ cleavage

3.5.1. 5-Iodo-2-methoxyphenol (3). The resin **1A** or **1B** or **1C** (100 mg, 0.5 mmol/g theoretical loading) was swelled with dry DCM for 30 min, $AlCl_3$ (10 equiv.) was added and the reaction mixture was shaken under Ar for 3 h. After this time the resin was filtered off and washed with DCM (5×5 ml). The filtrates were washed with a sat. aq. solution of NH_4Cl (1×15 ml) and H_2O (6×15 ml), dried ($MgSO_4$ anhydrous), and concentrated under reduced pressure to give 5-iodo-2-methoxyphenol (11 mg, 88%). Spectroscopic data were the same as in the example above.

3.5.2. Deprotected lamellarins. The resin **4B** (220 mg, 0.44 mmol/g loading) was swelled in dry DCM (3 ml) for 30 min and $AlCl_3$ (220 mg, 1.65 mmol, 15 equiv.) was added. The reaction mixture was stirred in a vibromatic shaker at 25 °C for 6 h. It was then filtered and washed with DCM, AcOEt and MeOH (5×10 ml, each), the organic solvent was evaporated. The residue was taken with a sat. aq. solution of NH_4Cl and extracted with ethyl acetate (5×20 ml), then washed with brine (1×30 ml). The organic fraction was dried and evaporated. The HPLC/MS [C18-APCI$^+$ using H_2O (5 mM $AcNH_4$): acetonitrile gradient 30–100% acetonitrile in 15 min] shown three lamellarin derivatives: lamellarin U, retention time 8.0 min, calcd 515.16; found 516.19 $[M+H]^+$; demethyllamellarin U, retention time 6.9 min, calcd 501.14; found 502.15 $[M+H]^+$, and lamellarin L, retention time 6.6 min, calcd 501.14; found 502.15 $[M+H]^+$. The crude product was purified by HPLC. Lamellarin U (4.5 mg, 9.2%), demethyl-lamellarin L (1 mg, 2.0%), and lamellarin L (1.5 mg, 3.1%) were obtained.

3.5.3. Lamellarin U. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.11 (d, J=1.9 Hz, 1H, H-2′), 7.02 (d, J=8.2 Hz, 1H, H-5′), 6.99 (dd, J=8.2, 1.9 Hz, 1H, H-6′), 6.94 (s, 1H, H-4), 6.73 (s, 1H, H-10), 6.70 (s, 1H, H-13), 6.69 (s, 1H, H-1), 5.69 (s, 1H, C3-O*H*), 5.66 (s, 1H, C3′-O*H*), 4.80 (m, 1H, H-8), 4.72 (m, 1H, H-8), 3.95 (s, 3H, $C^{4'}$-OCH_3), 3.87 (s, 3H, C^{11}-OCH_3), 3.51 (s, 3H, C^2-OCH_3), 3.37 (s, 3H, C^{12}-OCH_3); 3.08 (dd, J=6.6, 5.7 Hz, 2H, H-9); ^{13}C NMR (150 MHz, $CDCl_3$)[23] δ 151.4 (C-3′), 149.3 (C-11), 147.9 (C-12), 146.8 (C-4′), 145.8 (C-4a), 143.6 (C-2), 136.3 (C-13b), 129.2 (C-1′), 128.6 (C-14a), 127.1 (C-9a), 123.4 (C-6′), 120.5 (C-13a), 117.4 (C-2′), 114.9 (C-14), 111.1 (C-5′), 110.9 (C-10), 110.7 (C-14b),108.8 (C-13), 104.1 (C-1), 103.3 (C-4), 56.2 ($C^{4'}$-O*C*H_3), 55.8 (C^{11}-O*C*H_3), 55.5 (C^2-O*C*H_3), 55.0 (C^{12}-O*C*H_3), 42.8 (C-8), 28,6 (C-9). (+)-HRMS *m*/*z* 516.1683 (calcd for $C_{29}H_{26}NO_8$ $[M+H]^+$ 516.1166, Δ −4.8 ppm).

3.5.4. Lamellarin L. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.11 (d, J=1.9 Hz, 1H, H-2′), 7.02 (d, J=8.3 Hz, 1H, H-5′), 6.98 (dd, J=8.3, 1.9 Hz, 1H, H-6′), 6.93 (s, 1H, H-4), 6.79 (s, 1H, H-10), 6.68 (s, 1H, H-1), 6.67 (s, 1H, H-13), 5.70 (bs, 2H), 5.60 (bs, 1H), 4.78 (m, 1H, H-8), 4.70 (m, 1H, H-8), 3.96 (s, 3H, $C^{4'}$-OCH_3), 3.50 (s, 3H, C^2-OCH_3), 3.41 (s, 3H, C^{12}-OCH_3), 3.01 (m, 2H, H-9); ^{13}C NMR (150 MHz, $CDCl_3$)[23] δ 146.4 (C-3), 146.3 (C-3′), 146.2 (C-4′), 145.7 (C-11), 145.1 (C-12), 143.2 (C-2), 135.4 (C-13b), 128.3 (C-1′), 128.2 (C-14a), 127.4 (C-9a), 122.9 (C-6′), 119.8 (C-13a), 117.3 (C-2′), 114.0 (C-10), 111.9 (C-5′), 108.4 (C-13), 104.1 (C-1), 103.5 (C-4), 56.2 ($C^{4'}$-O*C*H_3), 55.5 (C^2-O*C*H_3), 55.1 (C^{12}-O*C*H_3), 42.2 (C-8), 28.2 (C-9). (+)-HRMS *m*/*z* 502.1503 (calcd for $C_{28}H_{24}NO_8$ $[M+H]^+$ 502.1502, Δ −0.1 ppm).

3.5.5. Demethyllamellarin U. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.04 (d, J=2.0 Hz, 1H, H-2′), 7.00 (d, J=8.0 Hz, 1H, H-5′), 6.94 (dd, J=8.0, 2.0 Hz, 1H, H-6′), 6.92 (s, 1H, H-4), 6.74 (s, 1H, H-13), 6.73 (s, 1H, H-10), 6.57 (s, 1H, H-1), 5.68 (s, O*H*), 5.65 (s, O*H*), 5.36 (s, O*H*), 4.72–4.80 (m, 2H, H-8), 3.97 (s, 3H, $C^{4'}$-OCH_3), 3.89 (s, 3H, C^{11}-OCH_3), 3.49 (s, 3H, C^2-OCH_3), 3.07 (m, 2H, H-9); ^{13}C NMR (150 MHz, $CDCl_3$)[23] δ 146.3 (C-11), 146.2 (C-4′) 146.2 (C-3), 145.2 (C-4a), 144.9 (C-3′), 143.8 (C-12), 142.9 (C-2), 135.2 (C-13b), 128.1 (C-14a), 126.1 (C-9a), 122.8 (C-6′), 120.6 (C-13a), 117.1 (C-2′), 115.7 (C-14), 113.1 (C-14b), 112.1 (C-13), 111.2 (C-5′), 110.3 (C-10), 104.1 (C-1), 103.2 (C-4), 55.9 ($C^{4'}$-O*C*H_3), 55.7 (C^{11}-O*C*H_3), 55.3 (C^2-O*C*H_3), 42.3 (C-8), 28.8 (C-9). (+)-HRMS *m*/*z* 502.1508 (calcd for $C_{28}H_{24}NO_8$ $[M+H]^+$ 502.1502, Δ −1.1 ppm).

3.6. Typical procedure for TFA cleavage of Wang resin

3.6.1. 5-Iodo-2-methoxyphenol (3). A solution of TFA in DCM (1:1, 2 ml) was added to the Wang conjugate phenol **1C** resin (56 mg, 0.71 mmol/g theoretical loading) and the mixture was shaken for 2 h at room temperature. The resulting suspension was filtered of, the same acid solution was added and the mixture was shaken for 2 h. This process was repeated two times. Finally the resin was washed several times with DCM. The filtrates were washed with H_2O (3×25 ml), dried ($MgSO_4$ anhydrous), and concentrated under reduced pressure to give a very clean 5-iodo-2-methoxyphenol (8 mg, 81%). Spectroscopic data were the same as in the example above.

3.6.2. Hydroxy protected lamellarins. Following the general procedure of cleavage with TFA described above, from **4C** (300 mg) a reaction crude was obtained. The HPLC/MS [C18-APCI$^+$ using H_2O (5 mM $AcNH_4$): acetonitrile gradient 30–100% acetonitrile in 15 min]

shown two different lamellarins derivatives: 2′-chloro-3-*O* isopropyllamellarin U retention time 11.07 min, calcd 591.17; found 592.23 [^{35}Cl M+H]$^+$, 594.23 [^{37}Cl M+H]$^+$, 3-*O*-isopropyllamellarin U retention time 11.3 min, calcd 557.20; found 558.30 [M+H]$^+$. The crude product was purified by HPLC. 2′-Chloro-3-*O*-isopropyllamellarin U (2 mg, 2% overall yield), and 3-*O*-isopropyllamellarin U (8.5 mg, 9%, overall yield) were obtained.

3.6.3. 3-O-Isopropyllamellarin U. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.11 (d, J=2.0 Hz, 1H, H-2′), 7.01 (d, J=8.3 Hz, 1H, H-5′), 6.99 (dd, J=8.3, 2.0 Hz, 1H, H-6′), 6.90 (s, 1H, H-4), 6.73 (s, 1H, H-10), 6.72 (s, 1H, H-13), 6.71 (s, 1H, H-1), 5.68 (bs, 1H, O*H*), 4.83 (m, 1H, H-8), 4.74 (m, 1H, H-8), 4.52 (hept, 1H, C^3-OC*H*$(CH_3)_2$), 3.94 (s, 3H, C$^{4'}$-OC*H*$_3$), 3.87 (s, 3H, C^{11}-OC*H*$_3$), 3.45 (s, 3H, C^2-OC*H*$_3$), 3.37 (s, 3H, C^{12}-OC*H*$_3$), 3.09 (m, 2H, H-9), 1.37 (d, 6H, C^3-OCH(C*H*$_3$)$_2$); ^{13}C NMR (150 MHz, $CDCl_3$)[23] δ 148.8 (C-11), 147.4 (C-12), 147.1 (C-3), 146.4 (C-2), 146.3 (C-4′), 146.1 (C-3′), 135.8 (C-13b), 128.6 (C-1′), 128.1 (C-14a), 126.5 (C-9a), 122.9 (C-6′), 120.1 (C-13a), 117.3 (C-2′), 114.7 (C-14) 111.2 (C-5′),110.9 (C-10), 110.3 (C-14b), 108.8 (C-13), 105.1 (C-1), 103.5 (C-4), 71.4 (C^3-O*C*H$(CH_3)_2$), 56.2 (C$^{4'}$-O*C*H$_3$), 55.8 (C^{11}-O*C*H$_3$), 55.4 (C^2-O*C*H$_3$), 55.0 (C^{12}-OC*H*$_3$), 42.3 (C-8), 28.6 (C-9), 21.7 (C^3-OCH(*C*H$_3$)$_2$). (+)-HRMS *m*/*z* 557.2067 (calcd for $C_{32}H_{31}NO_8$ [M]$^+$ 557.2050, Δ −3.0 ppm).

The presence of the unexpected 2′-chloro-3-*O*-isopropyllamellarin U was confirmed by ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.11 (s, 1H, H-6′), 7.07 (s, 1H, H-3′) 6.90 (s, 1H, H-4), 6.75 (s, 1H, H-10), 6.62 (s, 1H, H-13), 6.56 (s, 1H, H-1), 5.61 (bs, 1H, O*H*), 4.93 (m, 1H, H-8), 4.66 (m, 1H, H-8), 4.51 (hept, 1H, C^3-OC*H*$(CH_3)_2$), 3.96 (s, 3H, C$^{4'}$-OC*H*$_3$), 3.87 (s, 3H, C^{11}-OC*H*$_3$), 3.48 (s, 3H, C^2-OC*H*$_3$), 3.42 (s, 3H, C^{12}-OC*H*$_3$), 3.09 (m, 2H, H-9) 1.38 (d, 6H, C^3-OCH(C*H*$_3$)$_2$); ^{13}C NMR (150 MHz, $CDCl_3$)[23] δ 148.9 (C-11), 148.4 (C-5′), 147.1 (C-3), 147.4 (C-12), 146.9 (C-4′), 146.5 (C-2), 145,8 (C-4a), 135.9 (C-13b), 128.3 (C-14a), 126.6 (C-9a), 126.2 (C-1′), 119.9 (C-13a), 118.6 (C-3′)[24] 112.0 (C-6′),[25] 110.9 (C-10), 109.9 (C-14b), 108.2 (C-13), 104.7 (C-1), 103.3 (C-4), 71.3 (C^3-O*C*H$(CH_3)_2$, 56.4 (C$^{4'}$-O*C*H$_3$), 55.7 (C^{11}-O*C*H$_3$), 55.4 (C^2-O*C*H$_3$), 55.0 (C^{12}-OC*H*$_3$), 45.4 (C-8), 28.6 (C-9), 21.6 (C^3-OCH(*C*H$_3$)$_2$). (+)-HRMS *m*/*z* 592.1751 (calcd for $C_{32}H_{31}NO_8Cl$ [M+H]$^+$ 592.1738, Δ −2.2 ppm.

Acknowledgements

This work was partially supported by CICYT (PBQU2000-0235) and Pharma Mar (Madrid). P.C. thanks MEC (Spain) for a pre-doctoral fellowship.

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