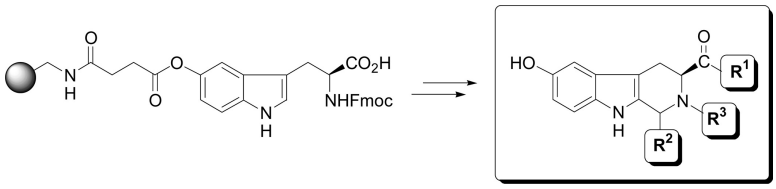
JOURNAL OF combinatorial CHEMISTRY

Article

Combinatorial Solid-Phase Synthesis of 6-Hydroxy-1,2,3,4-tetrahydro-β-carbolines from l-5-Hydroxytryptophan

Bruno Danieli, Paola Giovanelli, Giordano Lesma, Daniele Passarella, Alessandro Sacchetti, and Alessandra Silvani

J. Comb. Chem., **2005**, 7 (3), 458-462• DOI: 10.1021/cc049881m • Publication Date (Web): 16 March 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Journal of Combinatorial Chemistry is published by the American Chemical Society.
1155 Sixteenth Street N.W., Washington, DC 20036

Combinatorial Solid-Phase Synthesis of 6-Hydroxy-1,2,3,4-tetrahydro-β-carbolines from L-5-Hydroxytryptophan

Bruno Danieli, Paola Giovanelli, Giordano Lesma,* Daniele Passarella, Alessandro Sacchetti, and Alessandra Silvani

Dipartimento di Chimica Organica e Industriale e Centro Interdisciplinare Studi Biomolecolari e Applicazioni Industriali (CISI), Università degli Studi di Milano, Via G. Venezian 21, 20133 Milano, Italy

Received July 19, 2004

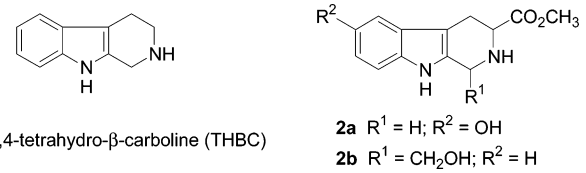
A library of biologically relevant 6-hydroxy-tetrahydro-β-carbolines (6-OH-THBCs) based on the L-5-OH-tryptophan scaffold was prepared. A solid-phase synthesis was developed, utilizing aminomethyl polystyrene resin and solid-phase-optimized reactions, such as Pictet−Spengler condensation. The library was designed such that three points of diversity would be readily introduced, making the strategy potentially suitable for generation of a large number of compounds.

Introduction

The 1,2,3,4-tetrahydro-β-carboline (THBC) core **1** (Figure 1) is a key structural motif found in a large class of tryptophan-derived alkaloids, which includes a number of complex examples such as yohimbine, ajmalicine, and the medicinally important reserpine.[1] Natural and synthetic products containing a β-carboline pharmacophore exhibit a wide range of important medicinal bioactivities, particularly concerning the central nervous system. Due to their unique rigid heterocyclic skeleton, many THBCs are known to bind with high affinity to benzodiazepine (BzR),[2] serotonin,[3] and dopamine[4] receptor sites and to inhibit monoamine oxidase A.[5] Particularly, the demonstration that certain β-carbolines potently inhibit [^{3}H]diazepam binding suggests this group of compounds as useful tools for studying benzodiazepine receptor as well as for the development of new therapeutic agents. The specific interactions of indolyl compounds containing this framework with BzR are strongly influenced by the presence of substituents on the polycyclic central unit. For instance, certain 3-methoxycarbonyl-tetrahydro-β-carbolines such as **2a** and **2b** were shown to exhibit potencies toward BzR comparable with that of clinically active benzodiazepines.[6]

The heterocyclic skeleton of THBCs possesses multiple sites for functionalization. Therefore, they are an ideal choice for the design of pharmacophore-based combinatorial libraries targeted at drug discovery, through generation of a large number of structurally diverse compounds.

Some combinatorial synthetic approaches have been developed to generate molecules containing the THBC core structure, both in liquid and in solid phase.[7] The synthetic strategy is mainly based on the acid-catalyzed Pictet−Spengler[8] condensation of tryptophan analogues with aliphatic and aromatic aldehydes. Yeh et al.[9] recently demonstrated the feasibility of parallel synthesis of THBCs on soluble poly(ethylene glycol) (PEG-OH) support. Wu and Schultz[10] reported a novel and versatile safety-catch linkage strategy for synthesis of highly derivatized β-carbolines on a polystyrene-derived resin. Starting from L-tryptophan immobilized on polystyrene resin, Ganesian and co-workers applied *N*-acyliminium Pictet−Spengler condensation to the synthesis of demethoxyfumitremorgin C analogues[11] and tetrahydro-β-carbolinehydantoin scaffolds.[12] A solid-phase synthesis of THBC-containing peptidomimetics was developed by Li et al.,[13] employing Fmoc amino aldehyde in the Pictet−Spengler condensation with a resin-bound tryptophan-containing fragment. In most of these approaches, a suitable *N*-protected tryptophan scaffold was attached to the resin via an ester linkage with the side chain carboxy group. This kind of strategy precluded the exploitation of the carboxy group as a possible site of diversity. Moreover, attempts to generate additional diversity by derivatizing the secondary nitrogen at position 2 of the THBC structure through reductive alkylation with aldehydes or reactions with acid halides, isocyanates, and sulfonyl chlorides were in many cases unsuccessful, probably due to sterical hindrance.[7f,k,13] We reasoned that the choice of a properly equipped L-tryptophan scaffold, bearing a further functional group for the loading on the solid support, could deliver significant benefits. The realization of this principle is shown in Scheme 1.



1 1,2,3,4-tetrahydro-β-carboline (THBC)

2a R^1 = H; R^2 = OH

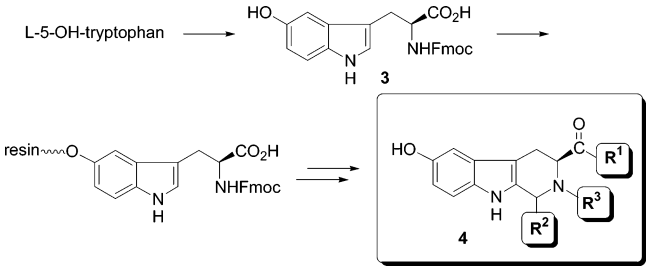
2b R^1 = CH_2OH; R^2 = H

Figure 1.

* Corresponding author. E-mail: giordano.lesma@unimi.it.

10.1021/cc049881m CCC: $30.25
Published on Web 03/16/2005

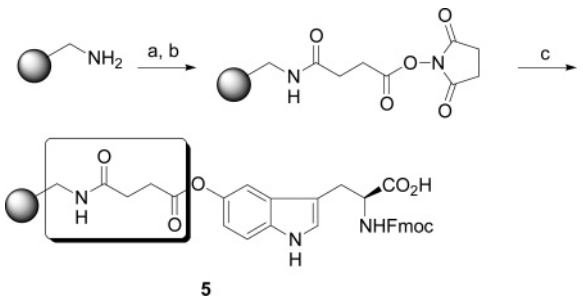
Scheme 1. General Strategy



The commercially available L-5-hydroxytryptophan, as *N*-Fmoc derivative **3**, could in principle be attached to the solid support through the phenol moiety, by means of a suitable bond that would allow protection of this functional group during the entire synthetic sequence. The resin-bound scaffold could be differently derivatized at the carboxylic acid group, thus introducing a first variable element R^1. After removal of the Fmoc group, access to the THBC skeleton could be realized through the Pictet−Spengler condensation, following solid-phase protocols. Due to the commercial availability of a wide variety of aliphatic and aromatic aldehydes, a large number of R^2 could in principle be proposed. At this stage, we are confident that subsequent reactions with various electrophiles (R^3) would be favored at the newly formed secondary nitrogen (N2) in the THBC structure due to the lower steric hindrance near the reaction center. Finally, cleavage of the template from the resin should afford the 6-OH-THBC derivatives **4**. Herein, we report the strategy for a solid-phase synthesis that allows easy introduction of three distinctive full diversity points, namely, at C1, N2, and carboxyl group of the carboline framework derived from L-5-OH-tryptophan. As proof-of-concept experiments, we have successfully applied this strategy to the synthesis of a 22-membered three-dimensional combinatorial library, which installs six, two, and four different R^1, R^2, and R^3 variables, respectively.

To load the scaffold **3** on the solid support, we chose to realize a phenolic ester linkage, which would be stable during the whole synthetic sequence, with particular regard to the acid-catalyzed Pictet−Spengler reaction. Moreover, this linkage should guarantee high loading yields and an easy cleavage of the final products under basic conditions. Recently, a novel serine-derived orthogonal linker[7i] has been proposed that allows phenol templates to be cleaved from the resin with fluoride ion. For instance, a **4**-type 6-OH-THBC-containing derivative (R^1 = OMe, R^2 = Ph, R^3 = $COCH_3$) was prepared in high yield and purity by treatment of *N*-Boc-L-5-OH-tryptophan methyl ester with a carbamoyl chloride derivative of serine. This step was followed by coupling with Tentagel-S-NH_2 resin (Rapp Polymer), Boc deprotection, acid-catalyzed Pictet−Spengler cyclization, and N2-acetylation. However, the application of this approach to the synthesis of a library of 6-OH-THBCs suffers from some major drawbacks. The carbamoyl chloride intermediate of the linker is incompatible with a 5-OH-tryptophan scaffold possessing an unprotected carboxylic group. Moreover, the synthesis of the linker itself is a multistep procedure, involving highly toxic starting materials.

Scheme 2. Loading of the *N*-Fmoc-L-5-OH-tryptophan Scaffold **3**[a]



[a] Reagents and conditions: (a) succinic anhydride, CH_2Cl_2, 12 h. (b) *N*-hydroxysuccinimide, DMAP, CH_2Cl_2, 3 h. (c) **3**, DMAP, CH_2Cl_2, DMF, 12 h.

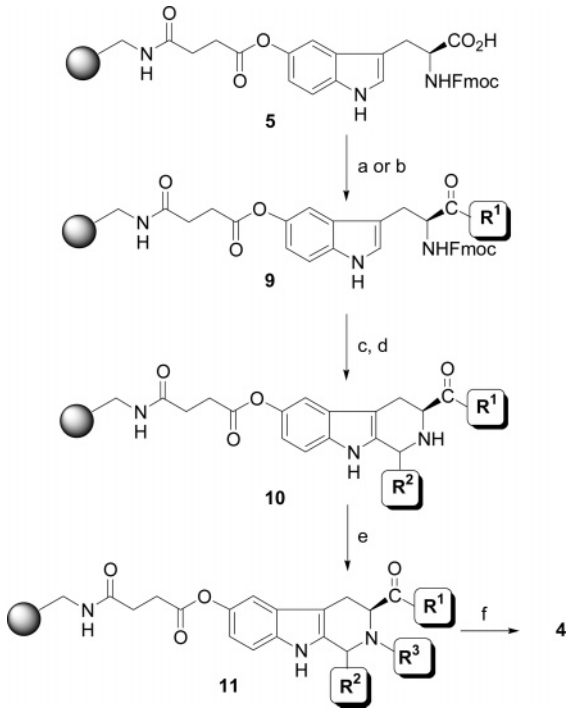
Results and Discussion

For the solid-phase procedure, we chose 1% cross-linked aminomethyl polystyrene (100−200 mesh, Nova-Biochem, loading: 1.42 mmol/g) as the solid support, suitably functionalized in order to obtain a supported carboxylic function. The synthetic sequence used for the preparation of resin bound substrate **5** is shown in Scheme 2. It allowed the loading of L-5-OH-tryptophan unprotected at the carboxylic group, thus preserving a possible site for developing diversity on the solid phase. The succinic acid moiety was introduced to the amino groups of the solid support by reaction with succinic anhydride in the presence of DMAP in CH_2Cl_2. The resulting carboxylic group was activated by reaction with *N*-hydroxysuccinimide and condensed with the scaffold **3**, still in the presence of DMAP in CH_2Cl_2. The resin-bound **5** was achieved with high substitution level (1.14 mmol/g, 80% loading), as estimated from elemental analysis data. The use of the Fmoc protective group enabled a quantitative confirmation of the loading percentage. In fact, when a sample of resin is deprotected, the resulting piperidine−dibenzylfulvene adduct can be quantified by its UV absorbance at 290 nm.[14]

The first element of diversity R^1 was introduced on **5** at this stage (Scheme 3), by amidation or esterification of the carboxylic group, under standard solid-phase conditions (2 equiv of amines **6**{*1*−*3*}[15] and 10 equiv of BOP, TEA, and DMF for amides; 10 equiv of alcohols **6**{*4*−*6*} and DIC, HOBT, and DMF for esters, see Table 1). As expected, the use in this step of amines produced a partial removing of the Fmoc protective group, which can be monitored by TLC control of the reaction medium. Anyway, this fact does not represent a problem since, in all cases, Fmoc had to be quantitatively cleared away by treatment of the resin with 10% piperidine in DMF.

The resin-bound free amine was then allowed to react with a 10 M equiv excess of aldehydes **7**{*1*−*2*} to afford resin-bound THBCs **10**, under Pictet−Spengler conditions. A solution of 1% TFA in dichloromethane at room temperature was found to be sufficient for this acid-catalyzed condensation without causing any side reaction on the scaffold. The Pictet−Spengler condensation was conveniently monitored by the Kaiser ninhydrin test, and the completion of the condensation step ensured when a negative test was observed.[16] Generally, the reaction was completed in 12 h.

Scheme 3. Solid-Phase Synthesis of 6-OH-1,2,3,4-Tetrahydro-β-carbolines[a]



[a] Reagents and conditions: (a) BOP, TEA, DMF, **6**{*1*−*3*} 2 eq, 12 h. (b) DIC, HOBT, DMF, **6**{*4*−*6*} 10 eq, 12 h. (c) 10% piperidine in DMF, 3 h. (d) **7**{*1*−*2*} 10eq, 1%TFA in CH_2Cl_2, 12 h. (e) **8**{*1*−*4*}, DIPEA, CH_2Cl_2, 6 h. (f) 35% ethylamine in water and THF (1:1), 3 × 30′.

Table 1. Sets of Reagents for the Library

6{*1*}	benzylamine	**7**{*1*}	benzaldehyde
6{*2*}	dimethylamine	**7**{*2*}	isovaleraldehyde
6{*3*}	piperidine	**8**{*1*}	di-*tert*-butyl dicarbonate
6{*4*}	cyclohexanol	**8**{*2*}	benzoyl cyanide
6{*5*}	benzyl alcohol	**8**{*3*}	*p*-toluenesulfonyl chloride
6{*6*}	methanol	**8**{*4*}	acetic anhydride

Formation of the condensation products **10** was confirmed for representative samples by cleavage from the resin followed by HPLC analysis[17] of the crude material. HPLC revealed the presence of both diastereisomers at C1 of the THBC skeleton in different ratio (up to 4:1). After chromatographic separation, the major diastereoisomer was determined to have the (1*R*, 3*S*) configuration (H1−H3 trans), in all examined cases, on the basis of literature data (^{13}C NMR[18] and CD[19]).

To install the R^3 diversity in our library, various kinds of derivatization of the secondary nitrogen at position 2 of **10** have been tested. Efficient acylation proceeded in the presence of benzoyl cyanide or acetic anhydride and of an excess of DIPEA, at room temperature in CH_2Cl_2.[20] Further functionalizations were performed by reaction with $(Boc)_2O$ and TsCl (DIPEA, CH_2Cl_2), to give the corresponding carbamate, and *p*-toluensulfonamide derivatives of type **11**. We could observe a high reactivity at N2, probably thanks to its distance from the sterically cumbersome solid support. Finally, cleavage of the product **11** from the resin was readily achieved (35% ethylamine in water and THF (1:1), room temperature, 30′ repeated three times[21]), providing the highly functionalized 6-OH-THBCs chemset **4** after aqueous wash. We chose ethylamine for its low boiling point since it can be removed by evaporation. In all, a total of 22 different 6-OH-THBCs were prepared, and the purity and identity of each product was assessed by direct analysis of the crude using LC−HR−MS (Table 2). All desired structures were unambiguously confirmed and determined to have varying degrees of purity (60−85%). A global yield ranging from 40 to 60% could be estimated for representative samples (see Experimental Section), which were purified by silica gel chromatography. In these cases, identity of products was further confirmed by ^{1}H NMR and EI−MS analyses.

Conclusion

In conclusion, we demonstrated a method for the solid-phase combinatorial synthesis of a 6-OH-THBCs library, furthermore amenable to a last modification on the hydroxyl group at C6. We have developed a strategy that allows facile installation of diversities not only at the C1 position of the carboline skeleton but also, more importantly, at the N2 and at the carboxylic group. By utilizing multiple steps of solid-phase reactions, this strategy permits the easy preparation of potentially large arrays of small molecules containing the important THBC pharmacophore.

Our ongoing efforts for the synthesis of related heterocyclic systems, through the key Pictet−Spengler reaction, will be reported elsewhere in due time.

Experimental Section

General. All chemicals were purchased from commercial sources and used directly, unless indicated otherwise. All chemical reactions were run under N_2, unless otherwise indicated. The ^{1}H and ^{13}C NMR spectra were taken on Bruker 300 and 400 MHz Avance NMR spectrometers. Chemical shifts are reported in parts per million downfield from $SiMe_4$ (δ 0.0). HPLC analyses were performed on a Jasco LC2000 series system. LC/HRMS analyses were run on a Finnigan LCQ Advantage MAX instrument.

Preparation of *N*-Fmoc-L-5-OH-tryptophan (3). To a stirred solution of 2.5 g (11.4 mmol) of L-5-OH-tryptophan in 50 mL of a 2:1 mixture of 10% aqueous Na_2CO_3/THF, a solution of FmocOSu (4.3 g, 12.5 mmol) in THF (20 mL) was added dropwise at room temperature. The reaction was stirred for 2 h. The reaction was made basic by addition of 20 mL of saturated Na_2CO_3 and was extracted with EtOAc. The aqueous layer was acidified to pH 2 with 1 M HCl and extracted with EtOAc. The organic extract was dried over Na_2SO_4, filtered, and concentrated in vacuo to yield a yellow oil. The oil was purified by flash chromatography on SiO_2 (5:95 MeOH/CH_2Cl_2) to give 4.1 g (82% yield) of **3**. ^{1}H NMR (300 MHz, DMSO-*d*) δ 10.5 (s, 1H), 8.23 (s, br, 1H), 7.78 (d, 2H, J = 8.3 Hz), 7.60−7.49 (m, 2H), 7.40−7.20 (m, 4H), 7.12 (d, 1H, J = 8.5 Hz), 6.98 (s, 1H), 6.90 (s, 1H), 6.78 (d, 1H, J = 8.5 Hz), 6.45 (s, br, 2H), 5.45 (d, 2H, J = 7.5 Hz), 4.50−4.32 (m, 3H), 4.21−4.10 (m, 1H), 3.40−3.25 (m, 2H). HRMS calcd for $C_{26}H_{22}N_2O_5$: 442.4756. Found: 442.4781.

Procedure for Loading of 3 on the Solid Support. 1. Preparation of resin 5. 1% cross-linked aminomethyl polystyrene (100−200 mesh, Nova-Biochem, loading: 1.42 mmol/g) resin (2.84 mmol) and succinic anhydride (14.2

Table 2. 6-OH-THBCs Chemset **4** Synthesized According to Scheme 3

6-OH-THBC	R^1	R^2	R^3	purity[a] **(%)**	yields (%)	HR MS (calcd)	HR MS (found)
4{*1,1,1*}	NHBn	Ph	Boc	68	44[b]	497.5993	497.5972
4{*1,1,3*}	NHBn	Ph	Ts	71	49[b]	551.6696	551.6704
4{*3,1,3*}	1-piperidyl	Ph	Ts	81	39[c]	529.6633	529.6625
4{*3,1,1*}	1-piperidyl	Ph	Boc	80	42[c]	475.5929	475.5938
4{*2,1,4*}[d]	NMe_2	Ph	Ac	75		377.4469	377.4452
4{*6,1,4*}	OMe	Ph	Ac	81	34[c]	364.4045	364.4037
4{*6,1,1*}	OMe	Ph	Boc	84	37[c]	422.4852	422.4840
4{*4,1,1*}	OCy	Ph	Boc	67	40[c]	490.6047	490.6071
4{*4,1,3*}	OCy	Ph	Ts	72	34[c]	544.6751	544.6762
4{*5,1,1*}	OBn	Ph	Boc	84	32[c]	498.5840	498.5846
4{*1,2,1*}[d]	NHBn	iBu	Boc	65		477.6089	477.6070
4{*3,2,2*}	1-piperidyl	iBu	Bz	74	41[c]	459.5935	459.5926
4{*1,2,2*}	NHBn	iBu	Bz	69	25[b]	481.5999	481.6012
4{*3,2,1*}	1-piperidyl	iBu	Boc	71	55[b]	455.6025	455.6037
4{*2,2,1*}	NMe_2	iBu	Boc	81	31[c]	415.5372	415.5378
4{*2,2,2*}[d]	NMe_2	iBu	Bz	82		419.5282	419.5299
4{*6,2,4*}	OMe	iBu	Ac	69	62[b]	344.4141	344.4128
4{*2,2,4*}	NMe_2	iBu	Ac	76	58[b]	357.4565	357.4560
4{*2,2,3*}	NMe_2	iBu	Ts	61	28[c]	469.6075	469.6052
4{*4,2,1*}	OCy	iBu	Boc	79	36[c]	470.6143	470.6134
4{*4,2,3*}	OCy	iBu	Ts	75	38[c]	524.6847	524.6861
4{*5,2,1*}	OBn	iBu	Boc	70	40[c]	478.5936	478.5925

[a] Determined by HPLC analysis.[17] [b] Isolated yield. [c] Estimated by HPLC analysis on the crude. [d] Not evaluated yields.

mmol) in CH_2Cl_2 (10 mL) were shaken overnight at room temperature. The resin was then filtered, washed with CH_2Cl_2 (3×) and again suspended in CH_2Cl_2 (10 mL). *N*-Hydroxysuccinimmide (14.2 mmol) and DMAP (5.68 mmol) were added, and the mixture was shaken for 3 h at room temperature. The resin was then filtered, washed with CH_2Cl_2 (3×), and again suspended in CH_2Cl_2 (10 mL). *N*-Fmoc-L-5-OH-tryptophan (5.68 mmol), DMAP (5.68 mmol), and DMF (3 mL) were added, and the mixture was shaken overnight at room temperature. The resin was then filtered and washed with CH_2Cl_2 (3×) to give resin **5**.

2. Determination of Resin Loading by Fmoc Analysis. *W* mg of resin **5** (e.g., ~10 mg) was weighed precisely into a test tube; 1.0 mL of 20% piperidine in DMF was added, and the tube was shaken for 30 min. v μL of the supernatant solution (e.g., 50 μL) was transferred to a 5 mL test tube and diluted to V μL (e.g., 1000 μL) with 20% piperidine in DMF. An UV blank of the 20% piperidine/DMF solution was first obtained. The supernatant diluted solution was analyzed by UV absorbance at $\lambda = 290$ nm ($\epsilon = 5800$). The obtained absorbance value A was then used to determine the resin loading, as follows:

$$\text{resin loading (mmol/g)} = (A \times V \times 10^3)/(\epsilon \times v \times W \times \text{cell length (cm)})$$

General Procedure for Solid-Phase 6-OH-THBCs Chemset (4) Synthesis. To a suspension of resin **5** (ca. 1 g, 1.14 mmol/g) in DMF (20 mL), BOP (10 equiv), TEA (10 equiv), and amine **6**{*1*−*3*} (2 equiv) (see Table 1 in the text) were added. Alternatively, DIC (10 equiv), HOBT (10 equiv), and alcohol **6**{*4*−*6*} (10 equiv) were added. After the mixture was allowed to react at room temperature overnight, the resin was filtered; washed with CH_2Cl_2 (3×), DMF (3×), and CH_2Cl_2 (3×); filtered; and dried. The resin **9** was deprotected with 10% piperidine in DMF (rt, 2 h). After usual workup (filtration, washing of the resin [CH_2Cl_2 (3×), DMF (3×), and CH_2Cl_2 (3×)], and drying before using in the next step), the resin was suspended in 1% TFA/CH_2Cl_2 (20 mL). The aldehyde **7**{*1*−*2*} (10 equiv) was added and the mixture was allowed to react overnight. If the ninhydrin test showed a positive result, then the procedure was repeated. After usual workup, the resin **10** was suspended in CH_2Cl_2 (15 mL); **8**{*1*−*4*} (10 equiv) and DIPEA (10 equiv) were added. After 6 h and usual workup, the resin **12** was treated with 35% ethylamine in water and THF (1:1) for 30′, filtered, and washed [CH_2Cl_2, DMF, and CH_2Cl_2]. The procedure was repeated three times to ensure complete cleavage of the product. The combined filtrate was diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ (3×), dried under Na_2SO_4, filtered, and concentrated. For representative samples, the resulting residue was purified by filtration through a plug of silica, eluting with 75:25 ethyl acetate−hexane, to obtain the purified 6-OH-THBCs **4**.

4{*1,1,1*}: yield 44%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.30 (s, br, 1H), 7.70 (s, br, 1H), 7.50−7.32 (m, 5H), 7.31−7.21 (m, 5H), 7.08 (d, 1H, $J = 8.5$ Hz), 6.79 (s, 1H), 6.73 (d, 1H, $J = 2.0$ Hz), 6.58 (dd, 1H, $J = 8.5, 2.0$ Hz), 5.53−5.38 (m, 2H), 5.30 (s, 1H), 4.10 (dd, 1H, $J = 9.5, 5.0$ Hz), 3.33 (dd, 1H, $J = 15.9, 5.0$ Hz), 2.96 (dd, 1H, $J = 15.9, 9.7$ Hz), 1.40 (s, 9H); MS (EI) *m*/*z* 497 (M^+), 397, 261, 185, 106, 91, 77, 57.

4{*1,1,3*}: yield 49%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.80 (s, br, 1H), 7.75 (s, br, 1H), 7.70 (d, 2H, $J = 8.1$ Hz), 7.50−7.30 (m, 5H), 7.30 (d, 2H, $J = 8.1$ Hz), 7.25−7.20 (m, 5H), 7.08 (d, 1H, $J = 8.5$ Hz), 6.79 (s, 1H), 6.72 (d, 1H, $J = 2.0$ Hz), 6.59 (dd, 1H, $J = 8.5, 2.0$ Hz), 5.53−5.38 (m, 2H), 5.30 (s, 1H), 4.11 (dd, 1H, $J = 9.6, 4.9$ Hz), 3.30 (dd, 1H, $J = 15.9, 5.0$ Hz), 2.95 (dd, 1H, $J = 15.9, 9.7$ Hz), 1.70 (s, 3H); MS (EI) *m*/*z* 481 (M^+), 551, 397, 263, 184, 155, 91.

4{*1,2,2*}: yield 40%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.70 (s, br, 1H), 7.85−7.32 (m, 11H), 7.08 (d, 1H, $J = 8.5$ Hz), 6.79 (s, 1H), 6.72 (d, 1H, $J = 2.0$ Hz), 6.60 (dd, 1H, $J = 8.5, 2.0$ Hz), 5.53−5.38 (m, 2H), 4.47 (dd, 1H, $J = 10.8, 5.8$ Hz), 4.10 (dd, 1H, $J = 9.8, 5.2$ Hz), 3.33 (dd, 1H, $J =$

15.9, 5.0 Hz), 2.98 (dd, 1H, J = 15.9, 9.3 Hz), 1.93−1.86 (m, 1H), 1.81−1.70 (m, 2H), 1.16 (d, 3H, J = 6.4 Hz), 0.96 (d, 3H, J = 6.4 Hz); MS (EI) *m*/*z* 481 (M^+), 376, 241, 185, 105, 91, 77, 57.

4{*3,2,1*}: yield 55%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.10 (s, br, 1H), 7.90 (s, br, 1H), 7.11 (d, 1H, J = 8.5 Hz), 6.71 (d, 1H, J = 2.0 Hz), 6.58 (dd, 1H, J = 8.5, 2.0 Hz), 4.47 (dd, 1H, J = 10.8, 5.8 Hz), 4.11 (dd, 1H, J = 9.6, 5.0 Hz), 3.56 (m, 2H), 3.40 (m, 2H), 3.33 (dd, 1H, J = 15.9, 4.8 Hz), 2.95 (dd, 1H, J = 15.5, 9.7 Hz), 1.93−1.86 (m, 1H), 1.81−1.70 (m, 2H), 1.67 (m, 2H), 1.58 (m, 4H), 1.40 (s, 9H), 1.16 (d, 3H, J = 6.6 Hz), 0.99 (d, 3H, J = 6.6 Hz); MS (EI) *m*/*z* 455 (M^+), 355, 270, 146, 84, 57.

4{*6,2,4*}: yield 62%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.10 (s, br, 1H), 7.80 (s, br, 1H), 7.08 (d, 1H, J = 8.5 Hz), 6.75 (d, 1H, J = 2.0 Hz), 6.59 (dd, 1H, J = 8.5, 2.0 Hz), 4.47 (dd, 1H, J = 10.8, 5.8 Hz), 4.11 (dd, 1H, J = 9.5, 4.9 Hz), 3.68 (s, 3H), 3.33 (dd, 1H, J = 15.9, 4.9 Hz), 2.95 (dd, 1H, J = 15.5, 9.5 Hz), 2.08 (s, 3H), 1.93−1.86 (m, 1H), 1.81−1.70 (m, 2H), 1.16 (d, 3H, J = 6.4 Hz), 1.00 (d, 3H, J = 6.6 Hz); MS (EI) *m*/*z* 344 (M^+), 301, 287, 245, 185, 149, 57.

4{*2,2,4*}: yield 58%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.50 (s, br, 1H), 7.50 (s, br, 1H), 7.07 (d, 1H, J = 8.5 Hz), 6.70 (d, 1H, J = 2.0 Hz), 6.56 (dd, 1H, J = 8.5, 2.0 Hz), 4.45 (dd, 1H, J = 10.5, 5.3 Hz), 4.10 (dd, 1H, J = 9.6, 5.0 Hz), 3.33 (dd, 1H, J = 15.9, 5.0 Hz), 3.02 (s, 3H), 2.96 (dd, 1H, J = 15.9, 9.5 Hz), 2.94 (s, 3H), 1.93−1.86 (m, 1H), 2.08 (s, 3H), 1.81−1.70 (m, 2H), 1.16 (d, 3H, J = 6.4 Hz), 0.99 (d, 3H, J = 6.6 Hz); MS (EI) *m*/*z* 357 (M^+), 314, 241, 185, 87, 43.

Acknowledgment. The authors thank Consorzio COMBIGEN for financial support.

References and Notes

(1) *The Alkaloids, Chemistry and Pharmacology*; G. A. Cordell, Ed.; Academic Press: New York, 1993; Vol. 43.
(2) Oakley, N.: Jones, B. *Eur. J. Pharmacol.* **1980**, *68*, 381.
(3) (a) Khorana, N.; Smith, C.; Herrick-Davis, K.; Purohit, A.; Teitler, M.; Grella, B.; Dukat, M.; Glennon, R. A. *J. Med. Chem.* **2003**, *46*, 3930−3937. (b) Audia, J. E.; Evrard, D. A.; Murdoch, G. R.; Droste, J. J.; Nissen, J. S.; Schenk, K. W.; Fludzinski, P.; Lucaites, V. L.; Nelson, D. L.; Cohen, M. L. *J. Med. Chem.* **1996**, *39*, 2773−2780.
(4) Abou-Gharbia, M.; Patel, R. U.; Webb, M. B.; Moyer, J. A.; Andree, T. H.; Muth, T. A. *J. Med. Chem.* **1987**, *30*, 1818.
(5) (a) Callaway, J. J.: Gynther, J.: Poso, A.: Vepsäläinen, J.: Airksinen, M. M. *J. Heterocycl. Chem.* **1994**, *31*, 431. (b) Ho, B. T. *J. Pharm. Sci.* **1972**, *61*, 821.
(6) Cain, M.; Weber, R. W.; Guzman, F.; Cook, J. M.; Barker, S. A.; Rice, K. C.; Crawley, J. N.; Paul, S. M.; Skolnick, P. *J. Med. Chem.* **1982**, *25*, 1081.
(7) For solid-phase Pictet−Spengler reaction, see: (a) Nielsen, T. E.: Diness, F.: Meldal, M. *Curr. Opin. Drug Discovery Dev.* **2003**, *6* (6), 801−814. (b) Kane, Tim R.; Ly, Cuong Q.; Dener, Jeffrey M. *Abstr. Pap. Am. Chem. Soc.* **2001**, *222*, 533-ORGN. (c) Groth, T.: Meldal, M. *J. Comb. Chem.* **2001**, *3*, 34−44. (d) Chou, Y.-L.: Morrissey, M. M.: Mohan, R. *Tetrahedron Lett.* **1998**, *39*, 757−760. (e) Fantauzzi, P. P.; Yager, K. M. *Tetrahedron Lett.* **1998**, *39*, 1291−1294. (f) van Loevezijn, A.; van Maarseveen, J. H.; Stegman, K.; Visser, G. M.: Koomen, G.-J. *Tetrahedron Lett.* **1998**, *39*, 4737−4740. (g) Sauerbrei, B.; Jungmann, V.: Waldmann, H. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 1143−1146. (h) Mayer, J. P.; Bankaitis-Davis, D.; Zhang, J.; Beaton, G.; Bjergarde, K.; Andersen, C. M.; Goodman, B. A.; Herrera, C. J. *Tetrahedron Lett.* **1996**, *37*, 5633−5636. (i) Mohan, R.; Chou, Y.-L.; Morrissey, M. M. *Tetrahedron Lett.* **1996**, *37*, 3963−3966. (j) Yang, L.; Guo, L. *Tetrahedron Lett.* **1996**, *37*, 5041−5044. (k) Kaljuste, K.: Undén, A. *Tetrahedron Lett.* **1995**, *36*, 9211−9214.
(8) (a) Cox, E. D.; Cook, J. M. *Chem. Rev.* **1995**, *95*, 1797−1842. (b) Whaley, W. M.: Govindachari, T. R. *Organic Reactions*; Adams, R., Ed,; John Wiley and Sons: New York, 1951; Vol. VI, p 151.
(9) Yeh, W.-B.; Lin, M.-J.: Sun, C.-M. *Tetrahedron Lett.* **2003**, *44*, 4923−4926.
(10) Wu, T. Y. H.: Schultz, P. G. *Org. Lett.* **2002**, *4*, 4033−4036.
(11) Wang, H.: Ganesan, A. *Org. Lett.* **1999**, *1*, 1647−1649.
(12) Bonnet, D.: Ganesan, A. *J. Comb. Chem.* **2002**, *4*, 546−548.
(13) Li, X.; Zhang, L.; Zhang, W.; Hall, S. E.: Tam, J. P. *Org. Lett.* **2000**, *2*, 3075−3078.
(14) Kay, C.; Lorthioir, O. E.; Parr, N. J.; Congreve, M.; McKeown, S. C.; Scicinski, J. J.; Ley, S. V. In *Biotechnology and Bioengineering* (*Combinatorial Chemistry*); Wiley: New York, 2000/2001; Vol. 71, p 110−118.
(15) When benzylamine was used, we noted a lowering of the total yield, probably due to partial cleavage of the substrate from the resin in this stage.
(16) Sarin, V. K.; Kent, S. B. H.; Tam, J. P.; Merrifield, R. B. *Anal. Biochem.* **1981**, *117*, 147−157.
(17) The analysis were performed on a RP18 column, eluant ACN/water = 3/1 with 0.5% TFA, 0.8 flow.
(18) Accordingly to the literature, in all the examined cases, the C1 and C3 carbon atoms of the major trans diastereoisomer appeared upfield shifted (2−6 ppm) with respect to the same carbon atoms of the minor cis diastereoisomer. See: ref 8a.
(19) Tóth, G.; Clauder, O.; Gesztes, K.; Yemul, S. S.: Snatzke, G. *J. Chem. Soc., Perkin Trans. 2* **1980**, 701−703 and cited references.
(20) When we tried to use benzoyl chloride or acetyl chloride as acylating agent, we observed a partial cleavage of the material from the solid support.
(21) We found that this procedure was necessary in order to preserve the stability of the final products, especially in the cases of the ester derivatives.

CC049881M