Bioorganic & Medicinal Chemistry Letters 20 (2010) 3431–3435

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Versatile synthesis of new cytotoxic agents structurally related to hemiasterlins

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ARTICLE INFO

Article history:
Received 15 February 2010
Revised 26 March 2010
Accepted 27 March 2010
Available online 1 April 2010

Keywords:
Hemiasterlin
Taltobulin
Ag_2O
Anticancer drugs

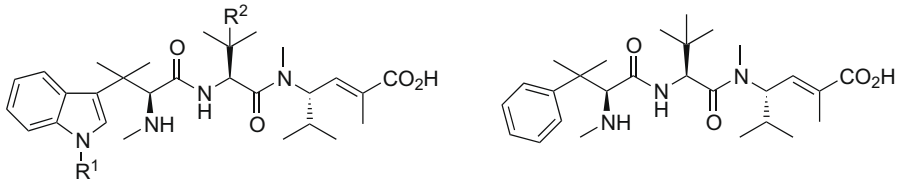
ABSTRACT

A representative series of structural analogues of the antimitotic tripeptides hemiasterlins have been synthesized. The key-step of this synthetic strategy consists of an Ag_2O-promoted nucleophilic substitution on a common precursor, a chiral non-racemic 2-bromoacyl derivative. Simple variation of nucleophile substituents allows a rapid and stereocontrolled development of new series of derivatives. Some reported compounds showed potent biological activity as growth inhibitors of cancer cell lines and tubulin polymerization inhibitors.

Hemiasterlins are members of the family of natural tripeptides, discovered and isolated from marine sponges some years ago,[1] Hemiasterlins contain three highly modified amino acids that are responsible for their stability and in vivo activity (Fig. 1). They are highly potent in suppression of microtubule depolymerization presumably by binding to the vinca alkaloid site of tubulin and cause mitotic arrest and cell death, thus making them very attractive molecules for new anticancer drugs.[2] In addition, a recent synthetic analogue of hemiasterlin, taltobulin (HTI-286, SPA-110), wherein a phenyl group replaces the 3-substituted indole ring, showed more potent in vivo cytotoxicity and antimitotic activity not only than the natural occurring tripeptides, but also in comparison with vincristine and paclitaxel.[3] Moreover, taltobulin appeared to be unaffected by resistance from P-glycoprotein drug transporter, and it has advanced into clinical trials.[4]

The synthesis of hemiasterlin was first reported in 1997 by Andersen et al. who also synthesized its potent derivative taltobulin in 2003, achieved by condensation of the three non-natural aminoacids **A**, **B** and **C** (Fig. 2).[5]

Among the three components, the **B** segment or L-*tert*-leucine is commercially available, whereas the **A** and **C** components must be synthesized from precursors. In particular, the enantioselective



$R^1 = R^2 = Me$: Hemiasterlin
$R^1 = H$, $R^2 = Me$: Hemiasterlin A
$R^1 = R^2 = H$: Hemiasterlin B
$R^1 = Me$, $R^2 = H$: Hemiasterlin C

Taltobulin (HTI-286, SPA-110)

Figure 1. Natural hemiasterlins and synthetic derivative taltobulin.

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doi:10.1016/j.bmcl.2010.03.098

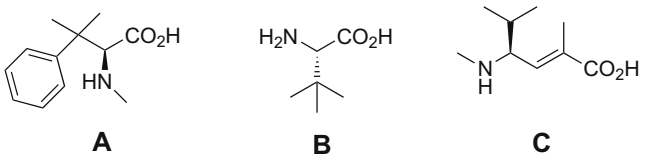


Figure 2. Synthetic building blocks for the achievement of taltobulin.

synthesis of **A** is very difficult, because the amine group must be introduced through a reductive amination with induction of chirality on C-α exploiting a transient Evans' oxazolidinone. On the other hand, SAR studies on compounds related to taltobulin and hemiasterlins suggest that their activity depends strongly on the presence of a α,α-dimethylbenzylic group on C-α of fragment **A** as well as a basic NHMe moiety located in the N-terminal position.[5c,6]

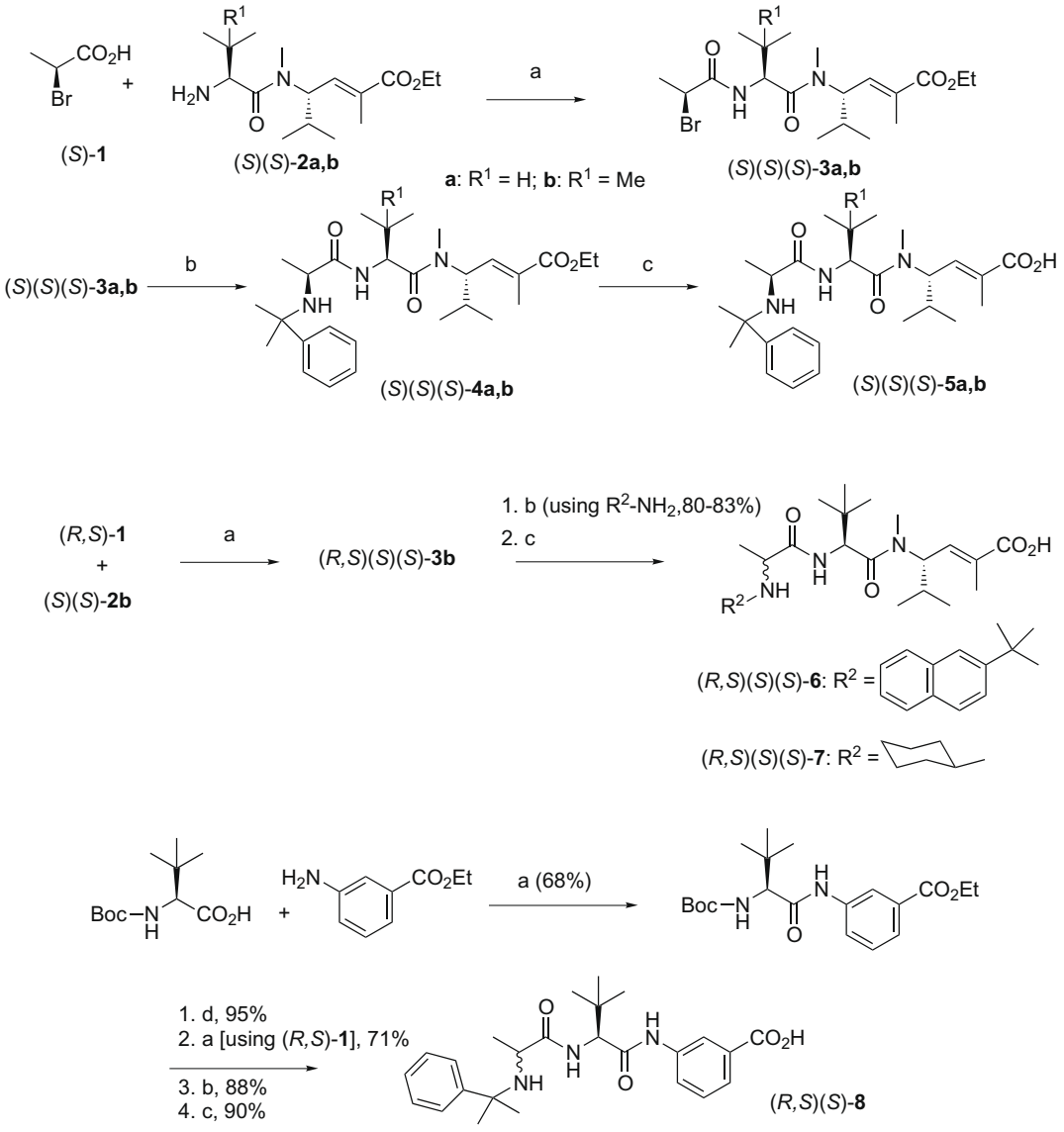
In this context, in order to expand our synthetic and biological studies on new anticancer tubulin polymerization inhibitors[7] and to explore new structural alteration of hemiasterlins, we have investigated the consequences of further modifications of fragments **A** and **C**.

We explored the possibility of finding a rapid way to build up a series of novel derivatives for SAR studies by exploiting common precursors to avoid de novo synthesis of particular fragment **A** synthons every time.

With this aim, a versatile enantioselective approach to synthesize novel classes of hemiasterlins that are structurally modified at fragment **A** is proposed here. This is based on a stereoselective substitution reaction in 2-haloamides promoted by Ag_2O that we have previously developed.[8] The same synthetic approach was also used to introduce different radicals at the N-terminal position of the tripeptide.[9]

Based on this approach, several novel pseudo-peptides bearing inverted position at the *gem*-dimethylbenzyl- and the methyl-groups on α-carbon and nitrogen with respect to N-terminus aminoacid of HTI-286 were designed and synthesized, and their preliminary biological activities were reported.

Alternatively, we expanded the procedure to different precursors, starting from both L-valine and L-*tert*-leucine as fragment **B**, as they represent a known variation allowing a substantial bioequivalence in literature. After linking the two intermediates 2-bromoacyl compounds with all three or only first two defined



Scheme 1. Reagents and conditions: (a) TMAC, DIPEA, THF, −78 °C (75–85%); (b) $PhC(Me)_2$–NH_2, Ag_2O, toluene (sonication, 90–92%); (c) LiOH/MeOH/water, then H^+ (85–90%); (d) trifluoroacetic acid (95%).

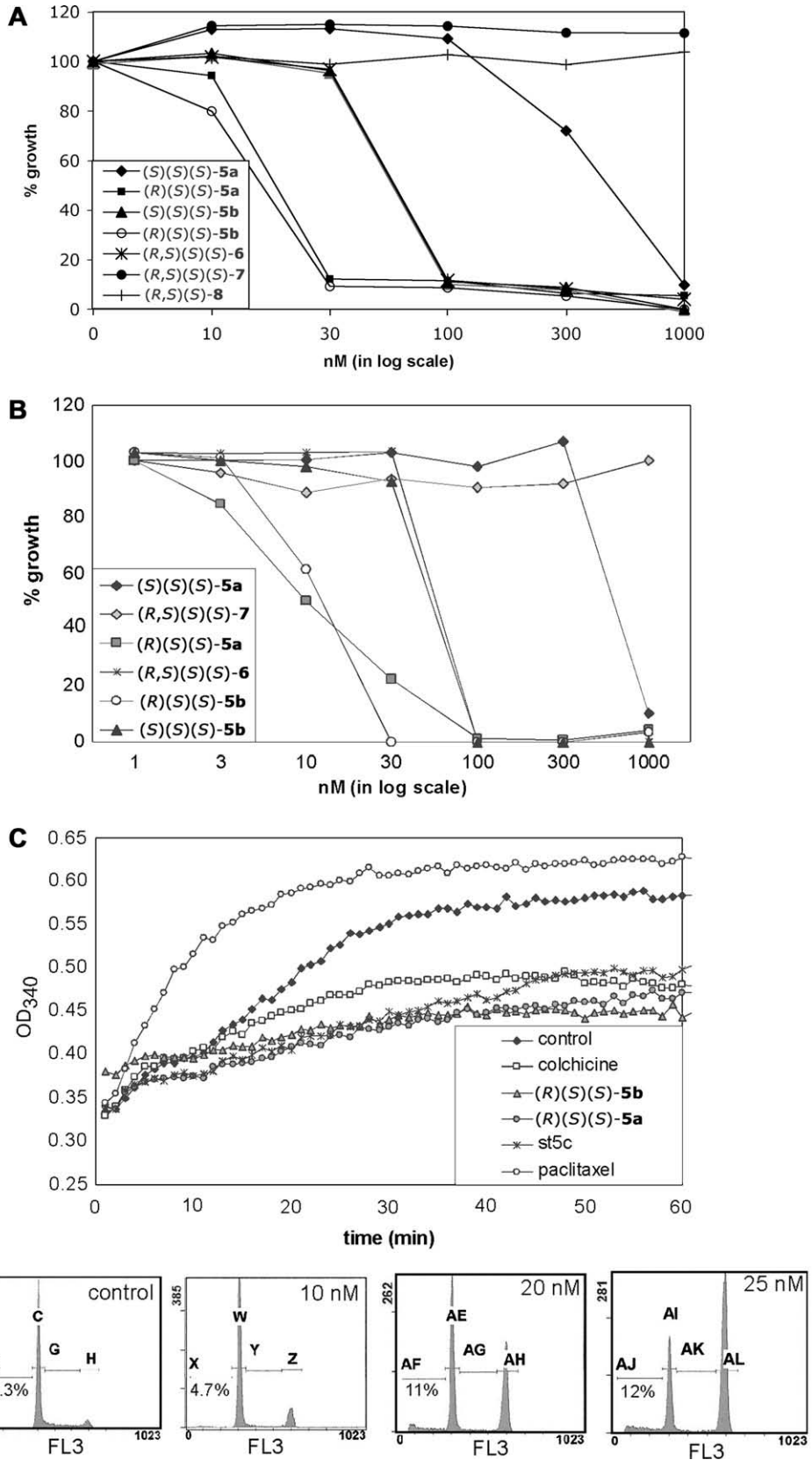


Figure 3. (A) UCI-101 cells were treated with various concentrations of compounds for 2 days. The tumor growth suppression was determined by Alamar blue staining and plotted against concentration of the treated drugs. Each concentration was repeated in triplicates. (B) Same studies were done with N1–S1 rat hepatoma cells. (C) Comparison of tubulin polymerization inhibition curves for compounds (*R*)(*S*)(*S*)-**5a** and **5b**, with those of colchicine (negative control for polymerization), *cis*-3,4′,5-trimethoxy-3′-aminostilbene (st5c) and paclitaxel (positive control for polymerization). (*R*)(*S*)(*S*)-**5a** and **5b** appear to be the most potent tubulin depolymerizers. All compounds are used at 5 μM concentration. Tubulin polymerization study was performed with the polymerization assay kit from Cytoskeleton (Denver, CO). (D) Cell cycle analysis was performed in UCI-101 cells after treatment with (*R*)(*S*)(*S*)-**5a** for 16 h. Cells were stained with ethidium bromide before FACScan analysis. Similar result was seen in (*R*)(*S*)(*S*)-**5b** but not shown.

chiral carbons, we were then able to produce a wide series of derivatives just by varying the nucleophile in the last reaction. In the present report, a limited representative set of derivatives are shown.

To obtain more information, we were also interested to expand modifications to fragment **C**. It is possible to synthesize a new series of more rigid derivatives replacing the alkene functional group with a cyclic aromatic ring, which can be easily achieved with an appropriate amino benzoic acid precursor.

Finally, for the purpose of exploiting the full potential of the stereoselective synthetic procedure, compounds **5a**,**b** were synthesized as single diastereomers but, for other derivatives, preliminary indications on activity were considered our main goal, and the synthesis of diastereomeric mixtures was preferred as being more informative.

Scheme 1 shows the synthesis of some target compounds via the key intermediates (*S*)(*S*)(*S*)-**3a**,**b**, which was conducted by adapting the literature procedure described for the fragments **BC**.[5] For clarity, only (*S*)(*S*)(*S*)-configuration are illustrated for **5a**,**b** but, in the same way, (*R*)(*S*)(*S*)-isomers were obtained using (*R*)-**1** as starting material.[10] For compound **8**, which bears the more rigid aromatic ring in place of fragment **C**, the intermediate **BC** was easily obtained through condensation of Boc-(L)-*tert*-leucine and 3-aminobenzoic acid ethyl ester.

More in detail, fragments **BC** [(*S*)(*S*)-**2a**,**b**] were condensed, via trimethylacetyl chloride (TMAC) at −78 °C, with (*S*)-2-bromopropanoic acid [(*S*)-**1**], in turn easily obtainable from (L)-alanine through a diazotization–bromuration reaction, to give the bromoacyl-peptides (*S*)(*S*)(*S*)-**3a**,**b**. In the next step, solid Ag_2O-promoted bromine displacement in key intermediates (*S*)(*S*)(*S*)-**3a**,**b** by appropriate nucleophiles, with full control of diastereoselectivity, giving *N*-alkyl peptides **4a**,**b** with retention of configuration. Interestingly, when the reaction was conducted with hindered nucleophiles as dimethyl benzyl amine, no substitution products were detected without the presence of Ag_2O, which becomes essential not only to obtain the desired stereochemistry. Finally, hydrolysis of esters **4a**,**b** yielded the expected derivates **5a**,**b** without configurational losses. Diastereomeric excesses of compounds **5a**,**b** were checked by NMR and HPLC analyses in comparison with diastereomeric mixtures obtained when starting from racemic (*R*,*S*)-**1** instead of (*R*) or (*S*)-**1**.[11] Using racemic (*R*,*S*)-**1**, compounds (*R*,*S*)(*S*)(*S*)-**6**,**7** and (*R*,*S*)(*S*)-**8** were similarly obtained as diastereomeric mixtures.

The cytotoxicity of each compound was examined in UCI-101 human ovarian cancer cells and N1–S1 rat hepatoma cells (Figs. 3A and B). The two most active compounds are (*R*)(*S*)(*S*)-**5a** and **5b** with IC_{50} of 20 nM, whereas (*R*,*S*)(*S*)(*S*)-**7** and (*R*,*S*)(*S*)-**8** have no cytotoxic activity even at 1 mM. The other two compounds (*S*)(*S*)(*S*)-**5b** and (*R*,*S*)(*S*)(*S*)-**6** have IC_{50} at 200 nM. The fact that (*R*)(*S*)(*S*)-**5a** and **5b** are the two most potent compounds compared with their stereoisomers (*S*)(*S*)(*S*)-**5a** and **5b** suggests the essential role of the first (*R*) configuration. With respect to taltobulin derivatives, in which (*S*)(*S*)(*S*) stereochemistry is reported to have potent activity, there is some discrepancy. However, the bulky dimethyl benzyl group in the most active diastereomers occupies the same place in both series. It seems, therefore, that the correct placement of dimethyl benzyl group is more important than the secondary amine to give functional interaction with binding site. The poor activity found with the aromatic 2-naphthyl-2-propyl group of **6**, suggests the presence of a large pocket that could be occupied by an aromatic group, similarly to the indole ring in natural parent compound hemiasterlin. Among the other substituents at nitrogen of fragment **A**, cyclohexyl did not lead to an active compound. Lack of the aryl portion also seems to be incompatible with growth inhibition, which is consistent with the results described in taltobulin series. Moreover, maintaining the fragment **A** as in compounds **5a**,**b**, the rigid modification at fragment **C** as in (*R*,*S*)(*S*)-**8** lead to loss of activity.

Similarly to other hemiasterlin derivatives, the most active compounds (*R*)(*S*)(*S*)-**5a** and **5b** were also tested for their effects on tubulin polymerization and cell cycle inhibition. In these studies, they demonstrated more potent activity to inhibit tubulin polymerization than the known natural tubulin inhibitors such as colchicine. Their activity is similar to the synthetic tubulin inhibitor *cis*-3,4′,5-trimethoxy-3′-aminostilbene (named st5c),[12] as comparison (Fig. 3C). In cell cycle analysis, moreover, both (*R*)(*S*)(*S*)-**5a** and **5b** induced cell cycle arrest at G2 phase, consistent with the fact that they are tubulin inhibitors. (*R*)(*S*)(*S*)-**5a** is shown in Figure 3D, with similar result in (*R*)(*S*)(*S*)-**5b**.

In summary, we here describe an efficient synthesis of novel hemiasterlins congeners. This very flexible procedure allows us to open a way to synthesize series of compounds where a number of **A** fragments may be synthesized from an α-bromoacid precursor, in turn available from any diazotizable α-aminoacid, and different nucleophiles. Further efforts to optimize the overall synthetic scheme and to generate a library of novel hemiasterlins are currently ongoing in the laboratory.

Acknowledgment

This work was financially supported in part by Ministero dell'Università e della Ricerca Scientifica e Tecnologica (PRIN 2006).

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10. *As sample procedures*: To a suspension of Ag_2O (464 mg, 2 mmol) in anhydrous toluene (5 mL), (*S*)(*S*)(*S*)-**3b** (446 mg, 1 mmol) and 2-phenyl-2-aminopropane (270 mg, 3 mmol) were added. The mixture was stirred by sonication under argon atmosphere for 1 h and then filtered over Celite. Evaporation to constant weight of the organic solution gave the crude product as a yellow oil that was purified by column chromatography (toluene/AcOEt 3.5:3) to give (*S*)(*S*)(*S*)-**4b** as a solid (451 mg, 90%). By comparison with TLC and NMR of diastereomeric mixture (*R*,*S*)(*S*)(*S*)-**4b** obtained starting from (*R*,*S*)(*S*)(*S*)-**3b**, we confirmed the optical purity of (*S*)(*S*)(*S*)-**4b**. ^{1}H NMR: δ 0.79 (d, 3H, J = 6.8 Hz), 0.87 (d, 3H, J = 6.8 Hz), 0.97 (s, 9H), 1.21 (s, 3H), 1.32 (t, 3H, J = 7.2 Hz), 1.40 (d, 3H,

J = 6.8 Hz), 1.52 (s, 3H), 1.87–1.91 (m, 1H), 1.90 (d, 3H, J = 0.8 Hz), 2.90–3.01 (br, 2H), 3.01 (s, 3H), 4.17–4.22 (m, 3H), 4.79 (d, 1H, J = 9.6 Hz), 5.08 (dd, 1H, J = 10.2 and 10 Hz), 6.63 (dd, 1H, J = 9.6 and 0.8 Hz), 7.19–7.49 (m, 5H). ^{13}C NMR δ 13.9, 14.3, 18.8, 19.5, 21.3, 26.5, 27.3, 30.0, 31.2, 32.2, 35.4, 54.7, 56.3, 61.0, 68.2, 124.6, 126.6, 128.4, 132.8, 138.2, 150.1, 167.8, 171.8, 174.6. To a solution of tripeptide ethyl ester (*S*)(*S*)(*S*)-**4b** (60 mg, 0.12 mmol) in MeOH (2 mL) and H_2O (1 mL), LiOH monohydrate (50 mg, 1.2 mmol) was added and the reaction mixture stirred for 3 h at rt. The mixture was acidified with TFA and then extracted with AcOEt (3 × 5 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuo to give (*S*)(*S*)(*S*)-**5b** as trifluoroacetate salt. The crude product was then purified by trituration with Et_2O (3 × 3 mL). Solid, (70 mg, 86%). ^{1}H NMR (CD_3OD) δ 0.77 d, 3H, J = 6.4 Hz, 0.86 d, 3H, J = 6.4 Hz, 0.90 s, 9H, 1.30 (d, 3H, J = 7.2 Hz), 1.75 (s, 3H), 1.80 (s, 3H), 1.84 (d, 3H, J = 0.6 Hz), 1.90–2.03 (m, 1H), 2.62 (d, 2H, J = 15.6 Hz), 3.03 (s, 3H), 3.80 (q, 1H, J = 7.2 Hz), 4.58 (s, 1H), 4.95 (dd, 1H, J = 10 and 9.6 Hz), 6.65 (dd, 1H, J = 9.6 and 0.6 Hz), 7.42–7.56 (m, 5H). ^{13}C NMR (CD_3OD) δ 13.4, 15.1, 18.6, 19.3, 19.5, 19.6, 26.0, 26.2, 28.7, 30.8, 34.7, 42.8, 52.1, 54.4, 55.8, 64.8, 72.1, 125.8, 127.6, 128.5, 131.2, 131.8, 138.3, 168.5, 171.2, 174.8. With a similar procedure, starting with (*R*)(*S*)(*S*)-**3b**, compound (*R*)(*S*)(*S*)-**5b** was obtained: ^{1}H NMR (CD_3OD) δ 0.89 (d, 3H, J = 6.8 Hz), 0.92 (d, 3H, J = 6.4 Hz), 0.96 (s, 9H), 1.39 (d, 3H, J = 6.8 Hz), 1.63 (s, 3H), 1.75 (s, 3H), 1.89 (d, 3H, J = 0.8 Hz), 2.01–2.10 (m, 1H), 3.09 (s, 3H), 3.78 (q, 1H, J = 6.8 Hz), 4.73 (s, 1H), 5.01 (dd, 1H, J = 9.8 and 9.4 Hz), 6.90 (dd, 1H, J = 9.0 and 0.8 Hz), 7.42–7.57 (m, 5H).^{13}C NMR δ 14.2, 18.9, 19.4, 19.9, 26.2, 26.8, 27.1, 31.1, 31.9, 35.6, 53.9, 57.9, 58.2, 64.4, 127.6, 130.6, 130.7, 134.0, 139.6, 139.9, 168.6, 171.0, 172.9.

11. Single diastereomeric compounds (*S*)(*S*)(*S*)- and (*R*)(*S*)(*S*)-**3a** were also achieved by column chromatography separation of the mixture (*R*,*S*)(*S*)(*S*)-**3a**, obtained starting from racemic **1**.
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