Diversity-Oriented Asymmetric Synthesis of Hapalosin: Construction of Three Small C9/C4/C3-Modified Hapalosin Analogue Libraries

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A flexible approach to the β-hydroxy γ-amino acid residue (fragment **C**) of hapalosin has been developed on the basis of the the regio- and diastereoselective Grignard reaction. The method allows the introduction of different side chains at the C9 of hapalosin. Asymmetric syntheses of hapalosin (**1a**), 9-homohapalosin (**1b**), 9-*i*-butyl-hapalosin (**1c**), 8-*epi*-hapalosin (*epi*-**1a**), and three small libraries diversified at C9 (3-member, $\mathbf{1L_3}$), C9/ C4 (9-member, $\mathbf{1L_9}$), or C9/C4/C3 (27-member, $\mathbf{1L_{27}}$) have been produced using this method.

Introduction

One of the most challenging problems in cancer chemotherapy is the development of multidrug resistance (MDR). That is, tumor cells which survive the initial therapeutic treatment often become resistant not only to the original therapeutic agent but also to other structurally unrelated drugs.[1] One of the principal mechanisms of MDR is the expulsion of structurally diverse drugs by the transmembrane ATPase P-glycoprotein (P-gp). Consequently, the development of anti-MDR agents that are able to potentiate the cytotoxicity of common antitumor drugs toward drug-resistant cells is highly desirable. In 1994, Moore and coworkers reported the isolation of hapalosin (**1a**, Figure 1), a cyclic depsipeptide, from the blue-green alga (cyanobacterium) *Hapalosiphon welwitschii* W. & G. S. West (Stigonemataceae),[2] which was shown to possess significant MDR-reversing activity. The important bioactivity exhibited by hapalosin, combined with its structural intrigue, has resulted in many synthetic endeavors to obtain this molecule,[3] and several total syntheses of hapalosin,[4] its analogues,[4d,e,i,j,5a−d] and mimetics[5e,f] have been reported. The synthesis of non-*N*-methyl hapalosin also allowed the MDR-reversing activity to be attributed to the major *s-cis* rotamer of hapalosin.[4c,d,g]

The structure−activity relationship studies (cf. Figure 2)[6] demonstrated that only two analogues incorporating protected 4-hydroxy-L-proline residues into the β-hydroxy γ-amino acid moiety of hapalosin (C-9 side chain modification) are more active than hapalosin itself.[5a,b] It thus allows us to assume that there is much room to improve the multidrug resistance reversing activity of hapalosin by modifying the C9 side chain of the β-hydroxy γ-amino acid moiety, namely,

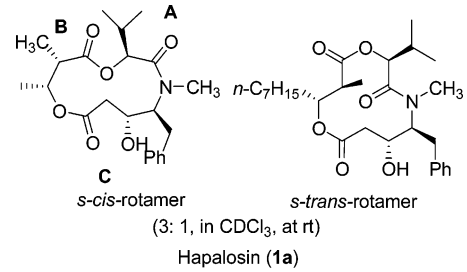

Figure 1. Two rotamers of hapalosin.

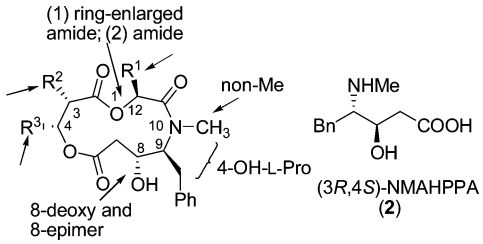

Figure 2. SAR studies performed for hapalosin analogues and the structure of the γ-amino-β-hydroxy acid residue of **1a**.

(3*R*,4*S*)-4-(*N*-methylamino)-3-hydroxy-5-phenyl-pentanoic acid (NMAHPPA) (**2**).[7]

All the retrosynthetic analysis of hapalosin converged at the disconnection of hapalosin into fragments **A** (α-hydroxy acid residue), **B** (β-hydroxy acid residue), and **C** (γ-amino-β-hydroxy acid residue).[4] While fragment **B** was conveniently synthesized[4,5] by asymmetric aldol reaction[8] using different chiral auxiliaries, diverse methods have been developed for the synthesis of γ-amino-β-hydroxy acid residue (fragment **C**) or its synthetic equivalents.[7] Nevertheless, the synthetic route to hapalosin and its analogues reported so far,[4] except that of Armstrong,[5a,b] did not involve modification at the β-hydroxy γ-amino acid moiety. Moreover, in most of the reported synthetic strategies of (3*R*,4*S*)-NMAHPPA (**2**),[4,7] the benzyl group was presented as an integral part of the starting materials such as (*R*)-phenylalanine. Such an approach does not allow for an easy variation

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of the side chain (benzyl group) and, thus, limits further structure−bioactivity relationship studies on this issue.

Combinatorial chemistry emerged in 1980s from peptide chemistry and has enjoyed rapid development to become a valuable approach in medicinal chemistry for lead searches and optimizations.[9,10] One of the keys to the combinatorial chemistry is the generation of the libraries by combination of diverse building blocks. The recognition of the fact that peptides do not normally make good drugs, combined with the development of chemical genetics and proteinomics have motivated, in last 10 years, organic chemists and medicinal chemists to focus on the construction of combinatorial libraries of small druglike organic molecules.[11] On the other hand, in addition to solid-phase synthesis, solution-phase synthesis has become an alternative technique in combinatorial chemistry.[12] Recent development in the field of proteinomics has stimulated the emergence of diversity-oriented organic synthesis.[13] Consequently, in addition to amide bond formation and related protection−deprotection procedures, the investigation of C−C bond-formation reactions, suitable for the generation of small organic molecule libraries, has become of primary importance. In this context, the use of C−C bond-formation reactions, including some modern organic reactions, such as the Heck reaction, Suzuki reaction, etc., in combinatorial chemistry have been accentuated.[14] However, not until recently has the use of the Grignard reaction, one of the most useful, routine, and versatile C−C formation reactions, in solution-phase combinatorial chemistry attracted attention.[15] In addition, the synthesis of a library of macrocycles[16] is a challenging yet important task in combinatorial chemistry.[17]

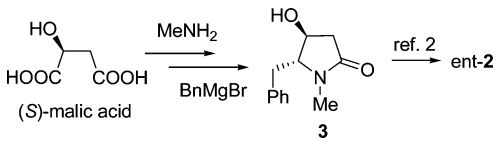
On the basis of these considerations and in combination with our ongoing program aimed at the development of malimides-based synthetic methodology,[18] we now report a diversity-oriented approach to hapalosin, its C9 homologue, C8 epimer, and three small analogue libraries focusing on the incorporation of a β-hydroxy γ-amino acid moiety bearing different C9 side chains.

Results and Discussion

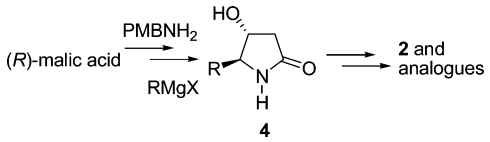
Our plan was to synthesize a series of hapalosin analogues bearing different substituents at the α-position of the γ-amino-β-hydroxy acid moiety. For this purpose, a flexible approach is required. Recent studies from these laboratories showed that protected (*S*)-malimides are suitable chirons for regio- and diastereoselective introduction of diverse alkyl groups via reductive alkylation.[18] The resulting 5-alkyl-4-hydroxy-2-pyrrolidinones, such as **3**,[19] can then be subjected to ring-opening conditions[2] to give the corresponding *N*-methyl-γ-amino-β-hydroxy acid (fragment **C**) (Scheme 1).

To synthesize hapalosin and its analogues, (*R*)-malic acid is the requisite enantiomer.[19b] In addition, C−C bond formation by Grignard reagent[14] addition with malimides

Scheme 1



Scheme 2



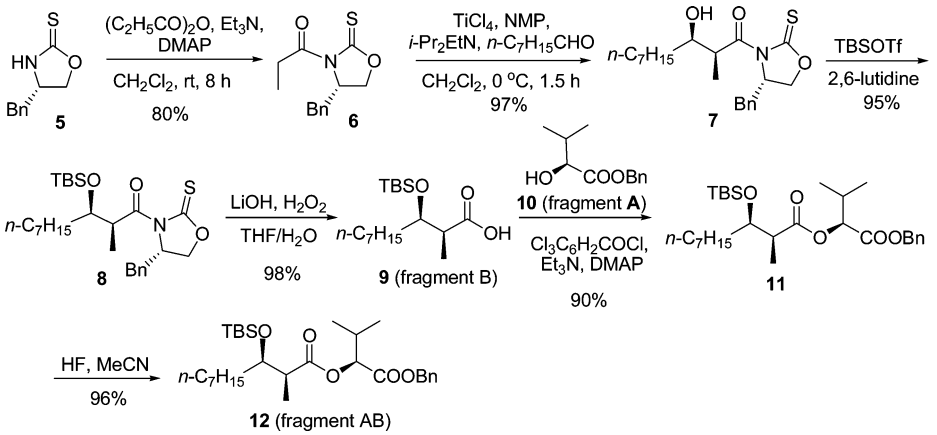
needs to be further studied for its applicability in diversity-oriented synthesis of hapalosin and its analogues (Scheme 2). Moreover, to make the method more flexible, a *p*-methoxybenzyl group[20] was selected as the *N*-protecting group that would allow changes to other *N*-alkyl groups.

The total synthesis of hapalosin started with the synthesis of fragment **AB** (**12**). Although the reaction sequence for the synthesis of fragment **B** described by Zhu[4g] works well, in searching for a more economic method, we sought to take advantage of recent advances[21,22] in the oxazolidine-2-thiones-based asymmetric aldol reaction. Crimmins's recent improvement[21a] over his initial report[21b] on the oxazolidine-2-thiones-based Evans *syn* diastereoselective aldol reaction allows the substitution of expensive Bu_2BOTf and (−)-sparteine[21b] with $TiCl_4$ and TMEDA, respectively. Thus oxazolidine-2-thione **5**, prepared in a 91% yield by the convenient method reported by Wu,[22] was propionated to give **6** in an 80% yield (Scheme 3). $TiCl_4$-mediated asymmetric aldol reaction of **6** with *n*-octanal under Crimmins conditions[21] gave **7** as the only observable diastereomer in a 97% yield. Protection of the C3 hydroxyl group by TBSOTf, followed by cleavage of the chiral auxiliary under Evans' conditions (LiOH, H_2O_2),[23] afforded fragment **B** (**9**), which was then coupled with fragment **A** (**10**)[24] to furnish compound **11**. Desilylation of **11** with HF in MeCN then provided fragment **AB** (**12**) in a 96% yield.

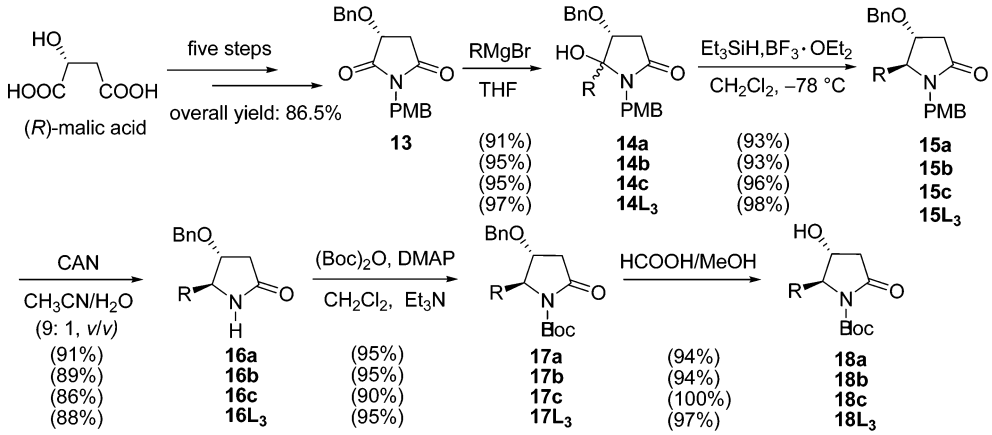
We next focused our attention on the synthesis of fragment **C**. The requisite (*R*)-malimide **13** was prepared from (*R*)-malic acid with minor modifications of the three-pot procedure[19] in an 87% overall yield (Scheme 4). Regioselective benzyl magnesium bromide addition, followed by diastereoselective reductive dehydroxylation of **13**, then gave successively **14a** and **15a** in 91 and 93% yields, respectively. The oxidative *N*-demethoxybenzylation under standard conditions[20] gave the desired **16a** in only a 60% yield. Gratefully, it was found that when a 9:1 mixture of MeCN/H_2O (v/v) was used as the mixed solvent, the yield was improved to 91%. The treatment of lactam **16a** with $(Boc)_2O$ gave the activated lactam **17a**, which was *O*-debenzylated to provide **18a**.

Lactam **18a** was smoothly converted to **19** under KCN-promoted nucleophilic ring-opening conditions (Scheme 5).[25] With the γ-amino-β-hydroxy acid ester **19** in hand, we investigated its *N*-methylation[26]. To this end, the hydroxyl group in **19** was first protected to give the corresponding silyloxy compound **20** in a quantitative yield. Saponification of **20** afforded **21** in an 84% yield. Unfortunately, in the presence of NaH, treatment of **21** with methyl iodide gave the desired *N*-methylated product **22** in a disappointing 35% yield. Since we were not able to improve the yield in the methylation step,[4f] an indirect method for the *N*-methylation[4g] was adopted.

Scheme 3

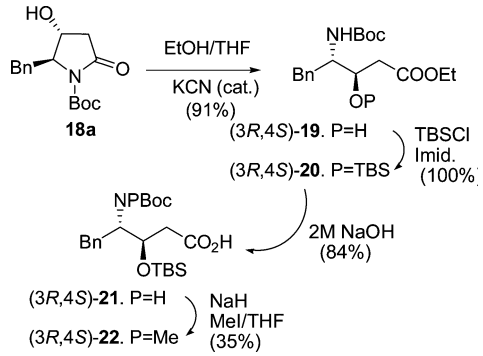


Scheme 4[a]



[a] R = Bn (**a**); CH_2Bn (**b**); *i*-Bu (**c**); *n*-C_3H_7, *n*-C_4H_9, *n*-C_5H_{11} (**L**$_3$).

Scheme 5



Thus, Zhu's procedure[4g] was adopted for the synthesis of hapalosin (Scheme 6). To this end, **19** was converted to **23**, in which the oxazolidinyl moiety was formed as a latent *N*-methyl group.[4g] Saponification of **23** with 1 N NaOH in ethanol gave **24a′**, which without separation was coupled with fragment **AB** (**12**) to give **25′** in a 76% yield. Compound **25′** was then converted to hapalosin (**1a**) in three steps without isolation of the intermediates. In this way, hapalosin (**1a**) was obtained in a 13% yield, along with a side product in a 20% yield (ratio of 1:1.6).

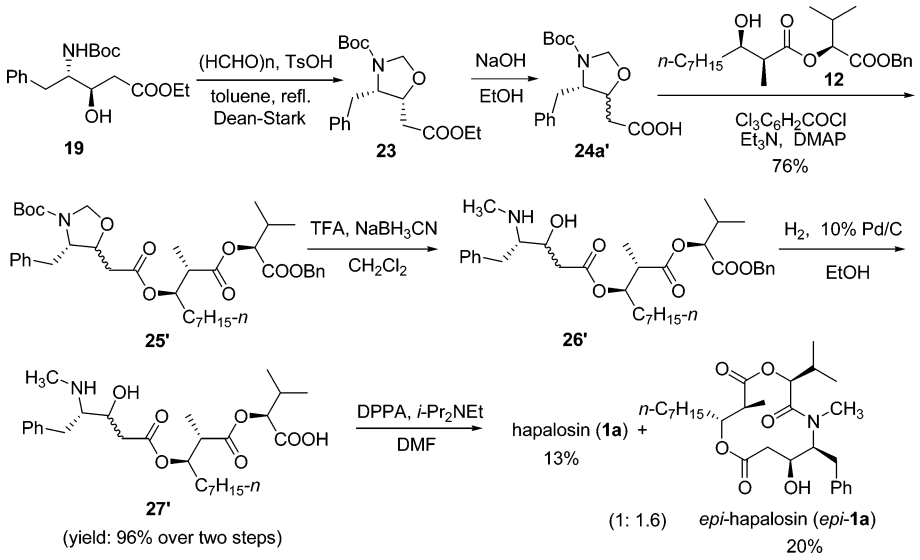
The analysis of the ^{1}H, ^{13}C NMR, and MS data suggested that the side product is an epimer of hapalosin (*epi*-hapalosin). This unexpected result prompted us to review the whole synthetic sequence. Thus, we isolated compound **27′**. The ^{1}H NMR spectral of **27′** clearly indicated the presence of two diastereomers in 1:1.6 ratio, which is in agreement with the observed ratio of hapalosin and its isomer. This confirmed that the byproduct isolated in the synthesis of hapalosin is indeed its epimer (*epi*-**1a**).

The next task was to determine at which step the epimerization occurred. From an inspection of the synthetic sequence and on the basis of mechanistic consideration, it appeared to us that the saponification step (**23** → **24a′**) was the most suspicious one. A plausible mechanism for the epimerization is a retro-Michael addition−intramolecular Michael addition under basic conditions (Scheme 7). The second step, namely, intramoleculer oxa-Michael addition, has been reported recently.[27]

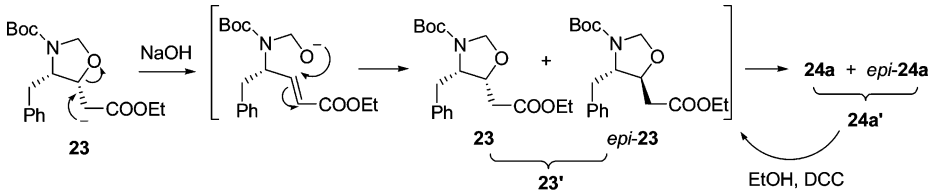
To further confirm this assumption, the saponification of **23** and the re-esterification of the resulting acid, **24a′**, were undertaken (Scheme 7). HPLC analysis of products **23′** revealed them to be a mixture of two diastereomers in a 1:1.6 ratio with the epimerized product (*epi*-**23**) being predominant (Figure 3).

Next, we sought to investigate the possibility of taking advantage of this epimerization to perform a stereodivergent[28] synthesis of the fragment **C**. As shown in Table 1, both saponification−re-esterification and base-promoted epimerization were investigated. While **23** is stable toward common organic bases (entries 5−7), under the saponification condi-

Scheme 6



Scheme 7



tions (aq NaOH), we observed the epimerization of **23**, and depending on the concentration of NaOH, the epimeric ratio varied from 1.3:1 to 2.3:1. A 1.3:1 ratio is approximately suitable for stereodivergent-oriented synthesis of hapalosin and its epimer.

To develop an epimerization-free synthesis of hapalosin, we elected to prepare the corresponding benzyl ester. Since we were unable to perform the ring-opening reaction of **18a** using benzyl alcohol as the nucleophile (cf. Scheme 5), the protocol displayed in Scheme 9 was investigated. Thus, treatment of **18a** with aqueous LiOH furnished, after acidic workup, the desired acid **28a** in high yield. Esterification of **28a** under classical conditions (BnBr, $NaHCO_3$, DMF, room temp, 36 h) furnished **29a** in an 89% yield. Oxazolidine **30a** was then formed under standard conditions ($(HCHO)_n$, *p*-TsOH, C_6H_6, reflux)[4g] and subjected to catalytic hydrogenolytic conditions (H_2, 10% Pd/C, EtOH, room temp, 2 h), which gave acid **24a** in a high overall yield.

For the coupling of fragment **AB** (**12**) with fragment **C** (**24a**), although Yamaguchi's conditions has been shown to give the desired product in a 72% yield,[4g] DCC turned out to be a superior coupling agent, which gave **31a** in an 89% yield (Scheme 10). The subjection of **31a** to ionic hydrogenation conditions (TFA, $NaBH_3CN$)[4g,29e] produced the *N*-methylated product **32a**, which was debenzylated to give **33a** in a 96% yield over two steps. Finally, diphenylphosphoryl azide (DPPA)-mediated[30] macrolactamization was undertaken, which furnished hapalosin (**1a**) in a 40% yield. The *s-cis/trans* rotameric ratio in $CDCl_3$ at room temp was estimated to be 3.00:1.55 on the basis of the resonances that appeared at δ 0.19 (d, J = 6.9 Hz, $3H_m$) and 0.22 (d, J = 6.9 Hz, $3H_M$), 0.57 (d, J = 6.9 Hz, $3H_M$) and 0.79 (d, J = 6.9 Hz, $3H_m$), or 2.86 (s, $3H_m$) and 2.86 (s, $3H_M$).

Table 1. Influence of Reaction Conditions on the Epimerization of Oxazolidine **23**

entry	base	equiv	*T*	*t*	*epi*-**23**/**23**[a]
1	0.5 N NaOH	10	room temp	30 min	2.1:1[b]
2	0.5 N NaOH	10	room temp	10 min	1.9:1[b]
3	1.5 N NaOH	30	room temp	10 min	1.3:1[b]
4	0.1 N NaOH	10	0 °C	4 h	2.3:1[b]
5	Et_3N	10	room temp	1 d	0:100[c]
6	pyridine	10	room temp	1 d	0:100[c]
7	*i*-Pr_2NEt	10	room temp	1 d	0:100[c]

[a] Ratio determined by HPLC. [b] Obtained by reaction conditions a of Scheme 8. [c] Obtained by reaction conditions b of Scheme 8.

Next, the method was extended to the total synthesis of hapalosin analogues. As shown in Scheme 11, the synthesis of 9-homo-hapalosin **1b** started with the reaction between phenylethyl magnesium bromide and malimide **13**, which was accomplished in an 80% yield. 9-Homo-hapalosin **1b**

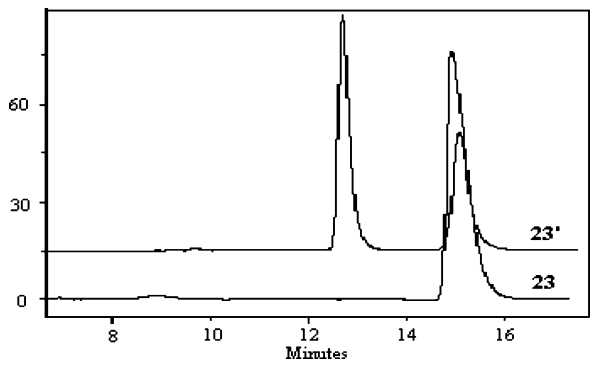
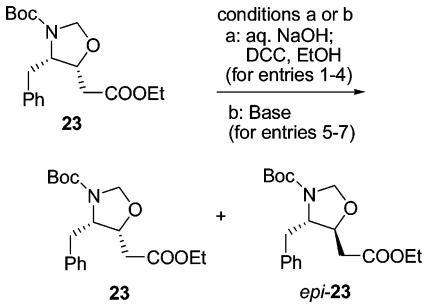
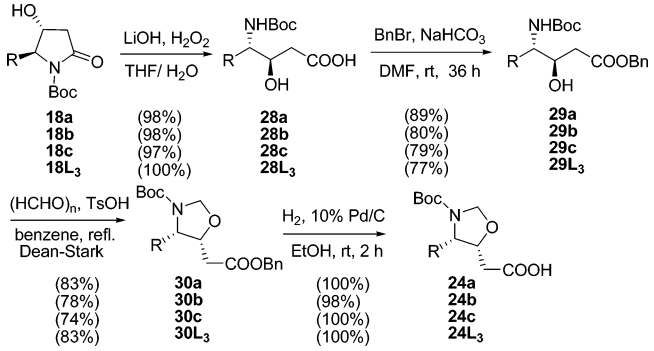


Figure 3. HPLC analysis of **23** and **23′**.

Scheme 8



Scheme 9[a]



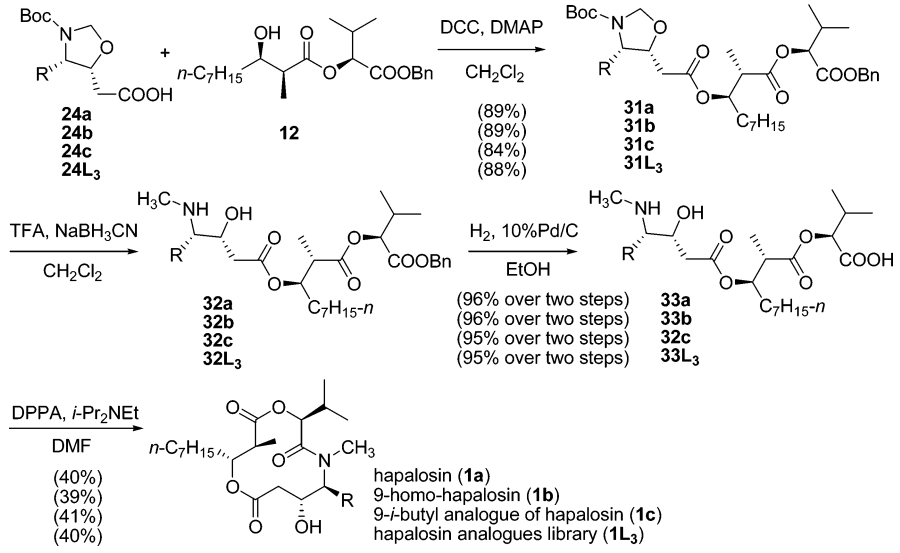
[a] R = Bn (**a**); CH_2Bn (**b**); *i*-Bu (**c**); *n*-C_3H_7, *n*-C_4H_9, *n*-C_5H_{11} (**L**$_3$).

was obtained, following the procedure described in Schemes 4, 9, and 10, in overall yields comparable to those of hapalosin. The result is promising because it showed that even with two Grignard reagents ($BnMgBr/BnCH_2MgBr$) with remarkable differences in reactivity, similar yields and regio- and diastereoselectivities were obtained in the Grignard reaction and subsequent reactions, leading ultimately to 9-homo-hapalosin **1b**. The rotameric ratio of homo-hapalosin **1b** in $CDCl_3$ at room temp was estimated to be 3.00:1.66, according to the integration of the resonances appeared at δ 2.72 (s, $3H_m$) and 2.85 (s, $3H_M$).

Because *i*-butyl is an important γ-substituent of statine (a *syn*-β-hydroxy-γ-amino acid), a key component of natural hexapeptide antibiotic pepstatin,[31] that was demonstrated to be a strong inhibitor of aspartic acid proteinases such as pepsin, renin, and cathepsin D[32] and because statine was reported to be an important part of a new class of triterpene derivatives with anti-HIV activity,[33] we decided to undertake the synthesis of a 9-*i*-butyl analogue of hapalosin (9-*i*-butyl-hapalosin), by substitution of the C9 benzyl group by *i*-butyl group. Following the procedure described in Schemes 4, 9, and 10, 9-*i*-butyl-hapalosin **1c** was obtained (Scheme 11), once again, in overall yields comparable to those of hapalosin. The rotameric ratio of 9-*i*-butyl-hapalosin **1c** in $CDCl_3$ at room temp was estimated to be 3.00:0.62, according to the integration of the resonances at δ 2.83 (s, $3H_M$) and 2.73 (s, $3H_m$).

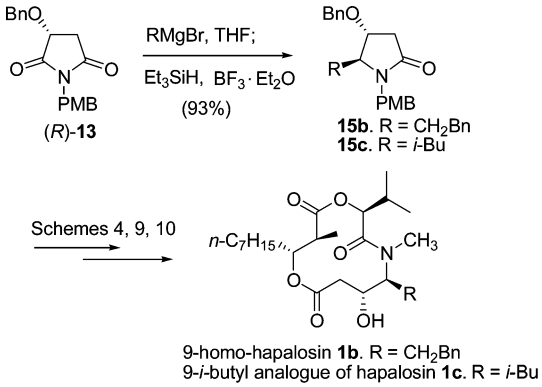
Now, the stage was set for the synthesis of hapalosin analogues libraries. The construction of a small three-member library was first investigated. Therefore, a 3-member Grignard reagent library (1.5 mol equiv in total), prepared by the treatment of an equal mole equivalent mixture of *n*-bromopropane, *n*-bromobutane, and *n*-bromopentane with an excess of magnesium, was subjected to a reaction with malimide **13**, which gave the *N*,*O*-acetals **14L$_3$** in a 97% yield (Scheme 12, cf. Scheme 4). The treatment of the *N*,*O*-acetals **14L$_3$** with BF_3-OEt_2/Et_3SiH yielded **15L$_3$** in a 98% yield. HPLC analysis of the resulting lactam library, **15L$_3$**, showed the presence of three components in a 35:33:32 ratio. Considering these results and those shown in the previous sections (Schemes 4 and 11), we assumed that each individual Grignard reagent, either alone or as a mixture in the Grignard reagent library **GL$_3$**, reacted with malimide **13** at about the same rate. Thus the reductive alkylation of malimide **13** is suitable for the construction of combinatorial libraries. Library **15L$_3$** was then converted, in seven steps, to **24L$_3$** (cf. Schemes 4 and 9), which was then coupled with fragment **AB** (**12**) to give **31L$_3$** (Scheme 10). Following the procedures described in Scheme 10, we converted **31L$_3$** into the 3-member library of hapalosin analogues **1L$_3$**. The presence of three hapalosin analogues in library **1L$_3$** was confirmed by both HPLC-MS analysis and HR-MS. All the

Scheme 10[a]

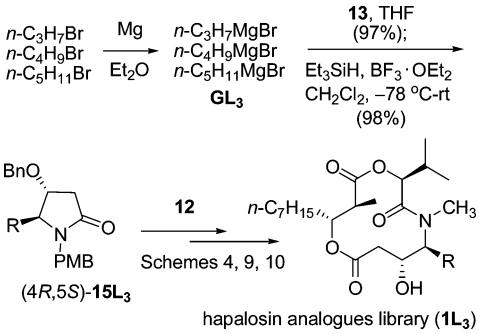


[a] R = Bn (**a**); CH_2Bn (**b**); *i*-Bu (**c**); *n*-C_3H_7, *n*-C_4H_9, *n*-C_5H_{11} (**L**$_3$).

Scheme 11. Asymmetric Synthesis of 9-Homo-hapalosin **1b** and 9-*i*-Butyl Analogue of Hapalosin **1c**



Scheme 12. Construction of a 3-Member Hapalosin Analogue Library (**1L$_3$**)[a]



[a] R = *n*-C_3H_7, *n*-C_4H_9, *n*-C_5H_{11}.

Table 2. Libraries Composition and Chemical Yields (3-Member Hapalosin Analogue Library **1L$_3$**) (Schemes 4, 9, 10, and 12)

library	**15L$_3$**	**16L$_3$**	**17L$_3$**	**18L$_3$**	**28L$_3$**[a]	**29L$_3$**
yield	98%	88%	95%	97%	100%	77%
ratio	35:33:32	33:33:33	33:33:33	31:33:35		33:32:33
library	**30L$_3$**	**24L$_3$**	**31L$_3$**	**32L$_3$**	**33L$_3$**[a]	**1L$_3$**
yield	93%	100%	88%	NC[b]	95% over two steps	40%
ratio	33:33:32	30:33:37	32:32:36			32:31:37

[a] Unable to perform a HPLC separation. [b] Used in the subsequence step without further characterization.

intermediate and final product libraries were characterized by IR, MS, ^{1}H NMR, and ^{13}C NMR analyses.

It is noteworthy that during the transformation of malimide **13** to the first 3-member hapalosin analogue library **1L$_3$** (Schemes 4, 9, 10, and 12), except for acids **28L$_3$** (Scheme 9) and **33L$_3$** (Scheme 10), all libraries show similar HPLC behaviors and were obtained in good component homogeneity (Table 2). The homogeneity of library **33L$_3$** can be seen by its conversion into library **1L$_3$** and from its ESI-MS spectrum (Figure 4), which shows roughly a 1:1:1 ratio.

Next, we envisioned the construction of a 9-member library, **1L$_9$**, by diversification of both fragment **B** and fragment **C** (Scheme 13, series a). Thus, Evan's chiral auxiliary-derived imide **34a** (R″ = Me) was subjected to asymmetric aldol reactions with a mixture of three aldehydes (*n*-hexanal, *n*-heptanal, *n*-octanal), and the desired aldol reaction products, **35aL$_3$**, were obtained in high combined

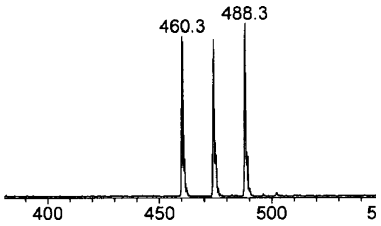
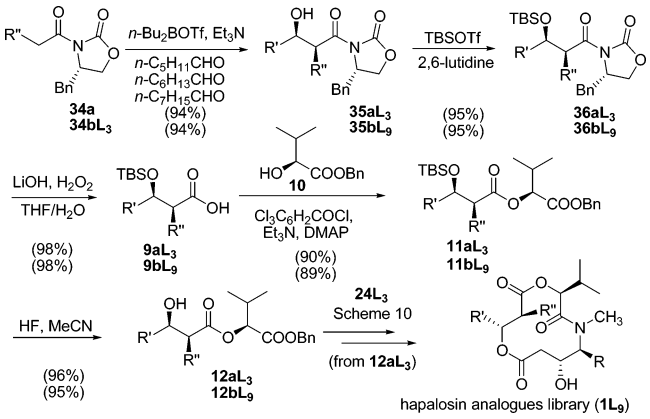


Figure 4. Partial (M + H$^+$) area of the ESI-MS spectrum of library **33L$_3$**.

Scheme 13. Construction of a 9-Member Hapalosin Analogue Library (**1L$_9$**)[a]



[a] R = *n*-C_3H_7, *n*-C_4H_9, *n*-C_5H_{11}; for series a, R″ = Me, and for series b, R″ = Me, Et, *n*-C_3H_7.

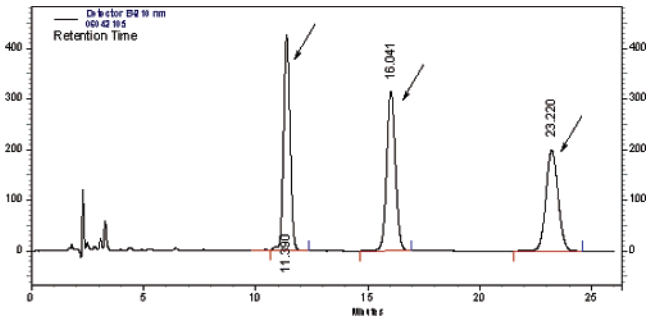


Figure 5. HPLC spectra of library **12aL$_3$** (ratio = 35:35:31).

Table 3. Library Compositions and Chemical Yields (9-Member Hapalosin Analogue Library (**1L$_9$**) (Schemes 13 and 10)

library	**35aL$_3$**	**36aL$_3$**	**9aL$_3$**[a]	**11aL$_3$**	**12aL$_3$**
yield	94%	95%	98%	90%	96%
ratio	34:34:32	35:34:30		33:35:32	35:35:31
library	**31L$_9$**	**32L$_9$**	**33L$_9$**[a]	**1L$_9$**	
yield	89%	NC[b]	95% over two steps	40%	
ratio	12:11:13:12: 12:11:11:10:10			11:12:12:12: 12:11:12:9:9	

[a] Unable to performed a HPLC separation. [b] Used in the subsequence step without further characterization.

yields. Subsequent reactions by the sequence depicted in Scheme 13 led to a library of fragment **AB-L$_3$** (**12aL$_3$**, Figure 5).

Next, following the same reaction sequence that was described for the synthesis of hapalosin (Scheme 10), we obtained a 9-member library of hapalosin analogues **1L$_9$** with each step in excellent yield. The presence of nine hapalosin analogues in the library **1L$_9$** was confirmed by HPLC and

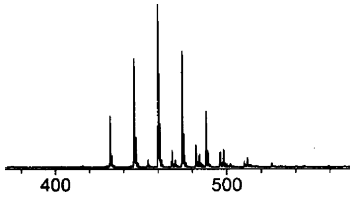


Figure 6. Partial ($M + H^+$) area of the ESI-MS spectrum of library **33L$_9$**.

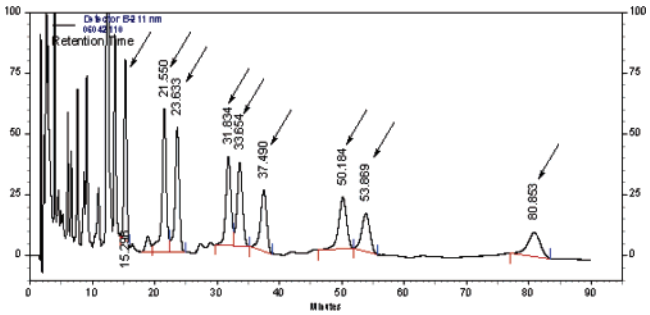
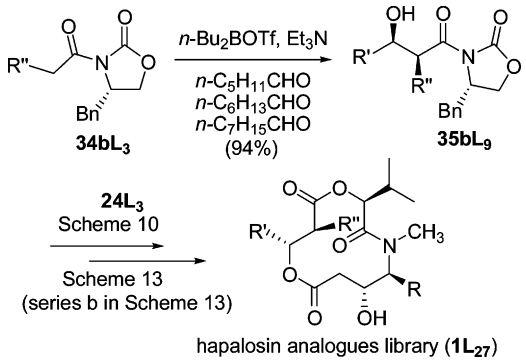


Figure 7. HPLC diagram of library **1L$_9$**.

Scheme 14. Construction of a 27-member Hapalosin Analogue Library (**1L$_{27}$**)[a]



[a] R = *n*-C_3H_7, *n*-C_4H_9, *n*-C_5H_{11}; R′ = *n*-C_5H_{11}, *n*-C_6H_{13}, *n*-C_7H_{15}; R″ = CH_3, C_2H_5, *n*-C_3H_7.

HR-MS. All the intermediate and final product libraries were characterized by IR, MS, ^{1}H NMR, and ^{13}C NMR analyses. The intermediate libraries formed during the synthesis of the 9-member hapalosin analogue library **1L$_9$**, except for acids **9L$_3$** (Scheme 13) and **33L$_9$** (Scheme 10), show similar HPLC behaviors and were obtained in good component homogeneity (Table 3). The homogeneity of library **33L$_9$** can be seen by its conversion into library **1L$_9$** and from its ESI-MS spectrum (Figure 6). As can be seen from Figure 6, many components share the same formula, and as a consequence, only five peaks were observed in the ESI-MS spectrum (the small peaks in this MS spectrum are $[M + Na]^+$ and $[M + K]^+$), which shows a ~1:2:3:2:1 ratio, corresponding to the mass population of the library.

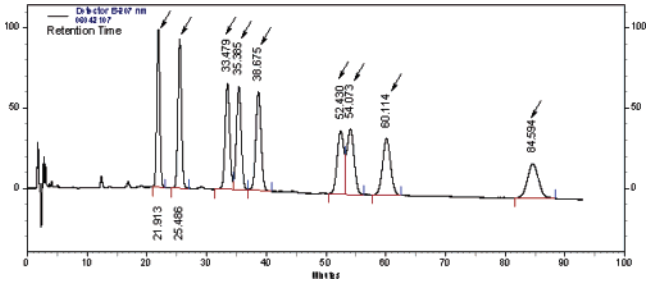


Figure 8. HPLC diagram of library **12bL$_9$**.

Finally, further variation the fragment **B** was pursued. Thus, a mixture of three aldehydes (*n*-hexanal, *n*-heptanal, and *n*-octanal) were subjected to reaction with a three-member library of imide **34bL$_3$** (Scheme 14; cf. Scheme 13, series b), which provided a library consisting of 9 components of aldol-products **35bL$_9$** that was then transformed into a library of fragment **AB-L$_9$** (**12bL$_9$**, Figure 8).

Coupling of library **12bL$_9$** with library **24L$_3$**, followed by the subsequent transformations depicted in Schemes 13 and 10, led to the formation of a 27-member library of hapalosin analogues **1L$_{27}$**. The presence of 27 hapalosin analogues in the library **1L$_{27}$** was confirmed by HR-MS analysis. All the intermediate and final product libraries were characterized by IR, MS, ^{1}H NMR, and ^{13}C NMR analyses. The intermediate libraries formed during the synthesis of the 27-member hapalosin analogues library **1L$_{27}$**, except for acids **9bL$_3$** (Scheme 13) and **33L$_{27}$** (Scheme 10), show similar HPLC behaviors and were obtained in good component homogeneity (Table 4). The low chemical yields in the last step and the overlap of the components prevent a good HPLC separation of the 27-member hapalosin analogues library **1L$_{27}$**; however, all components of **33L$_{27}$** were confirmed by HR-MS (Figure 9). As can be seen from Figure 9, many components share the same formula; as a consequence, only seven peaks were observed in the HR-MS spectrum, which shows a 1:3:6:7:6:3:1 ratio, corresponding to mass population of the library.

Conclusions

In summary, we have demonstrated that the reaction of multicomponent Grignard reagents[15c−e] with protected malimide gave, after subsequent reductive dehydroxylation, a uniform mixture of products and, thus, is suitable for the generation of substituted lactams **17**; on the basis of the reductive alkylation of malimide **13**, a variety of analogues of hapalosin fragment **C** (**24**) could be obtained. Coupling of such a fragment **C** library, **24L$_3$**, with fragment **AB** libraries **12aL$_3$**/ **12bL$_9$** allowed the preparation of hapalosin analogue libraries diversified at C9/C4/C3 in high component

Table 4. Libraries Composition and Chemical Yields (27-Member Hapalosin Analogues Library **1L$_{27}$**)

library	**35bL$_9$**	**36bL$_9$**	**9bL$_9$**[a]	**11bL$_9$**	**12bL$_9$**
yield	94%	95%	98%	90%	96%
ratio	14:11:14:11:10:11:11:10:8	13:14:12:12:12:10:10:10:8		12:13:11:11:12:10:11:11:9	11:12.5:11:12:12:10:11:11:9
library	**31L$_{27}$**	**32L$_{27}$**	**33L$_{27}$**[a]	**1L$_{27}$**[c]	
yield	89%	NC[b]	95%	40%	
ratio					

[a] Unable to perform a HPLC separation. [b] Used in the next step without further characterization. [c] Very complex HPLC diagram was obtained.

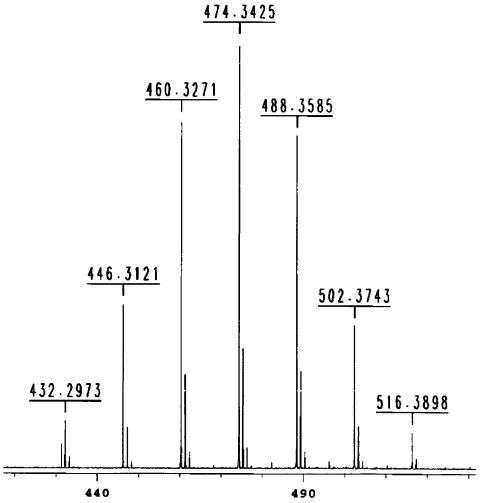


Figure 9. Partial ($M + H^+$) area of HR-MS spectrum of library $\mathbf{33L_{27}}$.

homogeneity at each individual step. In view of the higher MDR-reversing activity of the C9-modified analogues, the present method thus established a useful platform for searching more potent MDR-reversing agents.

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Supporting Information Available. Full experimental procedures and spectral data for all new compounds and libraries, ^{1}H NMR and ^{13}C NMR spectra of all new compounds and libraries, HPLC diagrams and MS/HR-MS spectra of selected libraries. This material is available free of charge via the Internet at http://pubs.acs.org.

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