A General Methodology for Automated Solid-Phase Synthesis of Depsides and Depsipeptides. Preparation of a Valinomycin Analogue†

Oliver Kuisle, Emilio Quiñoá, and Ricardo Riguera*

Departamento de Química Orgánica, Facultad de Química, and Instituto de Acuicultura, Universidad de Santiago de Compostela, E-15706 Santiago de Compostela, Spain

Received August 4, 1998 (Revised Manuscript Received July 21, 1999)

A general methodology is described that allows the solid-phase synthesis of depsides and depsipeptides from chiral α-hydroxy- and α-amino acids. The results of studies with different protecting groups for the α-hydroxy acids and coupling systems for depside bond formation are presented. The oligomers were prepared using a Wang-type linker with final TFA/CH_2Cl_2 cleavage. Depside linkage of the THP-protected acids (THP = tetrahydropyranyl) to the resin-bound chains was achieved with DIC/DMAP (DIC = diisopropylcarbodiimide, DMAP = 4-(dimethylamino)pyridine) and monitored by a color test with 4-(*p*-nitrobenzyl)pyridine. THP deprotection was achieved with *p*-TsOH in CH_2Cl_2/MeOH and was monitored by GC. Following the established procedure, depsides made up from the same enantiomer (i.e., H-[L-Man]$_8$-OH, **25**), by both enantiomers (i.e., H-[D-Man-L-Man]$_4$-OH, **26**), or by different hydroxy acids in the same chain (i.e., H-[L-Lac-L-Hiv]$_3$-OH, **27**) were prepared with an average yield of 95−97% per cycle. The linear precursor of the valinomycin analogue **30** ([L-Val-D-Man-D-Val-L-Lac]$_3$) was entirely synthesized on resin and cyclized in solution. Cyclization of the open-chain depsides is the final step in the preparation of a new class of chiral α-hydroxyester macrocycles.

Introduction

In recent years, the development of combinatorial chemistry[1] and the synthesis of a wide variety of nonpolymeric substrates[2] on resin has greatly increased the interest of researchers in the practical advantages of solid-phase synthesis. Since the pioneering work of Merrifield et al., this technique has become standard for the preparation of certain families of natural oligomers, such as nucleotides,[3] oligosaccharides,[4] and peptides.[5] However, no general solid-phase approach equivalent to that applied to peptides has been established for the preparation of depsides and depsipeptides, two structurally related classes of compounds that are present in terrestrial and marine sources.

Depsipeptides are oligomers that are similar to peptides but in which some of the amino acids are replaced by hydroxy acids, so that amide and ester bonds are present along the chain. One of the most representative examples of depsipeptides is the fungal antibiotic valinomycin (**1**),[6] a symmetric cyclodepsipeptide isolated from *Streptomyces fulvissimus*, which is formed by three repeating units of a sequence of four α-amino and α-hydroxy acids. It shows the best K^+/Na^+ selectivity of all K^+-ionophores known to date.[7] Onchidin B (**2**),[8] isolated from a tunicate from the South Pacific, is an example of a highly symmetric cyclodepsipeptide that contains α- and *β*-amino and hydroxy acids.

Both valinomycin and onchidin B share with many bioactive peptides a characteristic that explains the bioactivity of many of these compounds: they present a cyclic structure with the polar groups oriented toward the central cavity, whereas the rest of the molecule is relatively nonpolar. This enables them to complex ions and act as selective ion transporters through cellular membranes. A different biological mechanism has been identified for another type of cyclic depsipeptide, the quinomycins,[9] which are well-known DNA bisintercalators with potent antitumor activity.

Naturally, since ester and amide groups have certain common structural elements, but at the same time differ significantly in their hydrogen-bonding capacity, the incorporation of hydroxy acids into a peptide chain is a

* To whom correspondence should be addressed. Fax: + 34 981 591091.

† Abbreviations used for α-hydroxy acids and depsides are consistent with those used for α-amino acids and peptides in the nomenclature of peptides, with "H-" representing the hydroxy group and "−OH" representing the carboxy group: H-Hiv-OH = α-hyroxyisovaleric acid; H-Lac-OH = lactic acid; H-Man-OH = mandelic acid.

(1) Szostak, J. *Chem. Rev.* **1997**, *97*, 347−510.

(2) For reviews covering the period from 1992 to 1997, see: Booth, S.; Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees. D. C. *Tetrahedron* **1998**, *54*, 15385−15443, and the previous review cited therein.

(3) (a) Eckstein, F. *Nucleotides and Analogues: A Practical Approach*; Rickwood, D., Hames, B. D., Eds.; IRL Press: Oxford, 1991. For a recent review, see: (b) Beaucage, S. L.; Radhakrishnan, P. I. *Tetrahedron* **1992**, *48*, 2223−2311.

(4) For a review on earlier works in the field, see: Fréchet, J. M. J. *Polymer-Supported Reactions in Organic Synthesis*; Wiley: New York, 1980; Chapter 8, p 407. For more recent examples, see: (a) Seeberger, P. H.; Beebe, X.; Sukenick, G. D.; Pochapsky, S.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 491−493. (b) Nicolaou, K. C.; Winssinger, N.; Pastor, J.; DeRoose, F. *J. Am. Chem. Soc.* **1997**, *119*, 449−450.

(5) Stewart, J. M.; Young, J. D. *Solid-Phase Peptide Synthesis*, 2nd ed.; Pierce Chemical Co.: Rockford, IL, 1984.

(6) Dietrich, B.; Viout, P.; Lehn, J.-M. *Macrocyclic Chemistry*; VCH: Weinheim, 1993.

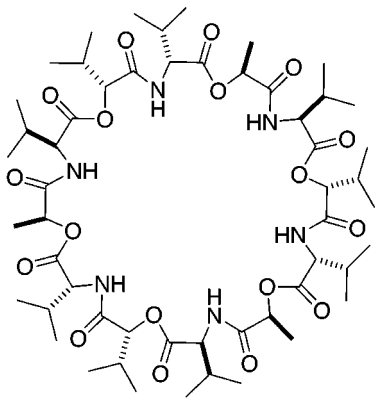
(7) Izatt, R. M.; Bradshaw, J. S.; Nielsen, S. A.; Lamb, J. D.; Christensen, J. J. *Chem. Rev.* **1985**, *85*, 271−339.

(8) Fernández, R.; Rodríguez, J.; Quiñoá, E.; Riguera, R.; Muñoz, L.; Fernández-Suárez, M.; Debitus, C. *J. Am. Chem. Soc.* **1996**, *118*, 11635−11643.

(9) Dell, A.; Williams, D. H.; Morris, H. R.; Smith, G. A.; Feeney, J.; Roberts, G. C. K. *J. Am. Chem. Soc.* **1975**, *97*, 2497−2506.

(10) Adang, A. E. P.; Hermkens, P. H. H.; Linders, J. T. M.; Ottenheijm, H. C. J.; van Staveren, C. J. *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 63−78.

10.1021/jo981580+ CCC: $18.00
Published on Web 10/04/1999



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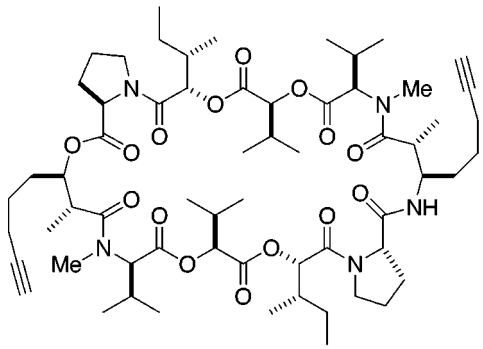

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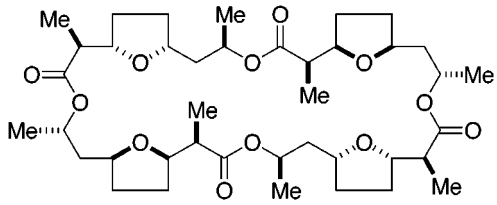


3

useful tool for the preparation of peptidomimetics[10] and for gaining a better understanding of their structural properties.[11]

For their part, the depsides are oligomers resembling peptides but are entirely built up by hydroxy acids rather than amino acids, and these hydroxy acids are connected through ester bonds. A representative example of naturally occurring depsides is nonactin (**3**), a member of the nactin family.[6] These macrocycles were isolated from actinomyces and all have the same cyclic structure formed by four units of an ω-hydroxy acid linked through ester bonds, and they differ only in the substitution pattern of the side chains.

Cyclic depsides derived from α-, β-, and γ-hydroxyacids have been isolated from the antibiotic extract of *Streptomyces fradiae*,[12] while some lichens produce the hexadepside H-(α-hydroxyisovalerate)$_6$-OMe.[13] For their part, polymeric substances derived from hydroxy acids such as poly[(*R*)-hydroxybutyrate][14] are known to be common storage materials in prokaryotic microorganisms, in which they can constitute up to 90% of the dry cell weight. In a lower molecular weight form, poly[(*R*)-hydroxybutyrate] has also been found in prokaryotic and eukaryotic cell walls, where it presumably participates in the formation of ion channels. Cyclic oligomers[15] of 3-hydroxybutyric acid and long chains of up to 128 units[16] have been prepared and studied by Seebach et al. Other synthetic polyesters derived from α- and β-hydroxy acids have gained considerable economic interest in the past few years and are produced on a large scale by biotechnological methods. These polymeric depsides show properties that are comparable to those of polypropylene but are accompanied by biodegradability and biocompatability, which makes them ideal as biodegradable substitutes for conventional plastics[17] or as surgical implants.[18]

Nevertheless, until now, no general and successful methodology for the solid phase synthesis of depside chains has been reported in the literature. Some solid-phase approaches have been described for the synthesis of depsipeptides, but these methods describe the introduction of hydroxy acids into a peptide chain. However, in these approaches either the ester bonds were created beforehand in solution and only the peptide bonds were formed on the resin[19] or the hydroxy acids were not protected at all[20] and the method relied on the greater reactivity of the amino group, which is a reasonable assumption when the substrate contains only one hydroxy acid. While our work was in progress, Davies et al.[21] proposed a combination of TBDMS (*tert*-butyldimethylsilyl) and Fmoc (9-fluorenylmethyloxycarbonyl) protection for the hydroxy and amino groups of a pentadepsipeptide synthesized on resin. However, the reported yields of 52% for this relatively small substrate, containing two standard peptide bonds and only two ester bonds, were relatively moderate and the monitoring of the completion of the ester bond formation so complex that its use for the synthesis of longer chains would probably become cumbersome.

In this paper, we wish to report our studies on the development of a general methodology for the creation of depsides and depsipeptides on solid support. The methodology gives high yields, ensures total preservation of the stereochemistry of the repeating units, and therefore permits the construction of long chains of α-hydroxy acids or combinations of α-hydroxy and α-amino acids.[22] The automated solid-phase synthesis of a depsipeptide analogue to valinomycin is described.

(11) (a) Seebach, D.; Ciceri, P. E.; Overhand, M.; Jaun, B.; Rigo, D. *Helv. Chim. Acta* **1996**, *79*, 2043−2066. (b) Yang, J.; Gellman, S. H. *J. Am. Chem. Soc.* **1998**, *120*, 9090−9091. (c) Gallo, E. A.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 9774−9788.

(12) Takeda, Y.; Masuda, T.; Matsumoto, T.; Takeshi, Y.; Shingu, T.; Floss, H. G. *J. Nat. Prod.* **1998**, *61*, 978−981.

(13) Bruun, T. *Phytochemistry* **1976**, *15*, 1261−1263.

(14) Müller, H.-M.; Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 477−502.

(15) Plattner, D. A.; Brunner, A.; Dobler, M.; Müller, H.-M.; Petter, W.; Zbinden, P.; Seebach, D. *Helv. Chim. Acta* **1993**, *76*, 2005−2033.

(16) Lengweiler, U. D.; Fritz, M. G.; Seebach, D. *Helv. Chim. Acta* **1996**, *79*, 670−701, and references therein.

(17) Doi, Y. *Microbial Polyesters*; VCH: Weinheim, 1990.

(18) *Polymers of Biological and Biomedical Significance*; Shalaby, S. W., Ikada, Y., Langer, R., Williams, J., Eds.; ACS Symposium Series 540; American Chemical Society: Washington, DC, 1994.

(19) For a recent example, see: Byung, H. L. *Tetrahedron Lett.* **1997**, *38*, 757−760.

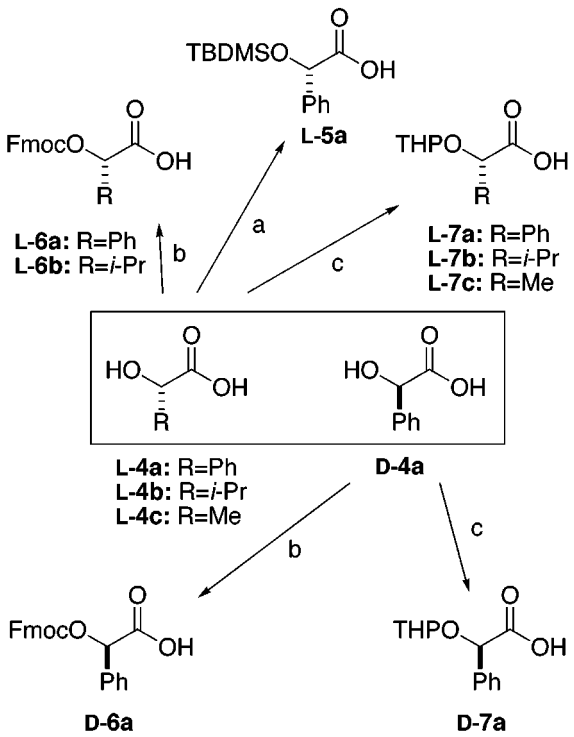
(20) For a recent example, see: Miyashita, M.; Nakamori, T.; Akamatsu, M.; Murai, T.; Miyagawa, H.; Ueno, T. *Pept. Chem.* **1996**, *34*, 137−140.

(21) Davies, J. S.; Howe, J.; Jayatilake, J.; Riley, T. *Lett. Pept. Sci.* **1997**, 441−445.

(22) For a brief account of these results, see: Kuisle, O.; Quiñoá, E.; Riguera, R. *Tetrahedron Lett.* **1999**, *40*, 1203−1206.

(23) Neises, B.; Steglich, W. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 522−524.

Scheme 1[a]



[a] Key: (a) (1) TBDMSCl (2.2 equiv), imidazole (2.4 equiv), THF, 3 h; (2) K_2CO_3 in THF/H_2O/MeOH (1:1:3), 2 h; (b) FmocCl (1.2 equiv), pyridine, THF, 15 h; (c) DHP (1.4 equiv), *p*-TsOH cat., $CHCl_3$, 2 h.

Results and Discussion

Assessment of Coupling Conditions and Protection. First of all, model coupling reactions were carried out in solution to find the most suitable protection for the hydroxy group of the α-hydroxy acids. Mandelic acid (**4**) was chosen as a monomer for these experiments because both enantiomers are commercially available. The O^{α}-protected L-mandelic acid derivatives **5a**−**7a** were prepared (Scheme 1) and submitted to depside coupling (DIC/DMAP,[23] THF, rt) with L-mandelic acid methyl ester (**8a**). Once we had decided which protecting group to use, we started a second series of experiments in order see if other coupling systems were superior to DIC/DMAP and compatible with the protective group. The results of these studies are described below.

Hydroxyl Protection. In our choice of a suitable protection method for the hydroxy group of the α-hydroxy acids we had to take into account the limited stability of the ester bonds, the possibility of racemization if basic conditions were to be used, and compatibility of the deprotection conditions with the resin linkage. We decided to work with a Wang-type linker because of the relatively smooth, acidic cleavage conditions, and so the range of possible protective groups was even more limited. For these reasons, we chose our protecting group among those that can be cleaved under mild acidic or basic conditions.

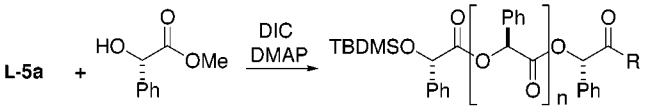
The first protecting group to be tested was the TBDMS group. Protection was accomplished using an excess of TBDMSCl to protect both the hydroxyl and the acid function of mandelic acid. The TBDMS ester was easily hydrolyzed to give O^{α}-TBDMS-L-mandelic acid (**5a**) in 89% yield. When **5a** was submitted to coupling with **8a**, as shown in Scheme 2, a rather complex mixture of products was obtained (Table 1). When this mixture was submitted to semipreparative HPLC analysis, the desired product, didepside **9**, was isolated in 38% yield along with substantial amounts of the tri- (**10**, 17%) and tetradepsides (**12**, 4%) and other derivatives, mainly the *N*-acylurea-derived side products **11** and **13**. This means that the TBDMS group was not sufficiently stable under the esterification conditions used for coupling and was therefore abandoned.

Table 1. HPLC Analysis of Coupling Reaction between 8a and 5a

compd	R	n	t_R[a]	% yield (%)
9	OMe	0	10 min 05 sec	38
10	OMe	1	13 min 45 sec	17
11	*N*-acylurea	0	17 min 35 sec	10[b]
12	OMe	2	20 min 10 sec	4
13	*N*-acylurea	1	26 min 25 sec	4[b]

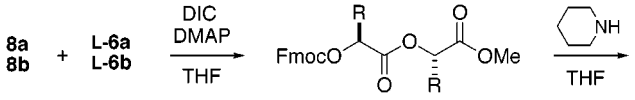
[a] HPLC conditions: μ-Porasil (7.8 • 300 mm), hexane/EtOAc (9:1), 2.0 mL/min. [b] The yields for the *N*-acylurea derivatives are based on the amount of **5a** (1.2 eq), since they are formed from **5a** and not **8a**.

Scheme 2

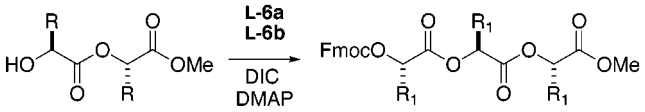


8a **9-13**

Scheme 3



14a: R=Ph
14b: R=*i*-Pr



15a: R=Ph
15b: R=*i*-Pr

16a: R=Ph
16b: R=*i*-Pr

Limited stability of this protecting group might also provide an explanation for the difficulties encountered by Davies et al. in their synthesis of the dolastatin D analogues.[21]

As Fmoc has been widely used to protect amino groups, we investigated its use to protect the hydroxy group of α-hydroxy acids. Thus, **6a** was prepared directly from **4a** with FmocCl/pyridine and was subsequently purified by crystallization (58% yield). The Fmoc-protected tridepside **16a** could be easily prepared by subsequent coupling−deprotection−coupling (Scheme 3). Nevertheless, when we tried to deprotect **16a** using the same conditions as before, two products were obtained that could be separated by HPLC and were apparently diastereomers resulting from racemization of one of the three mandelic acid units: in the ^1H NMR spectrum, both of the compounds showed three signals in the region of the H_{α} proton and the same molecular mass in LRFAB-MS. Intrigued by the fact that one racemization product was observed almost exclusively (assuming that the other one was the "intact" deprotected product) even though three

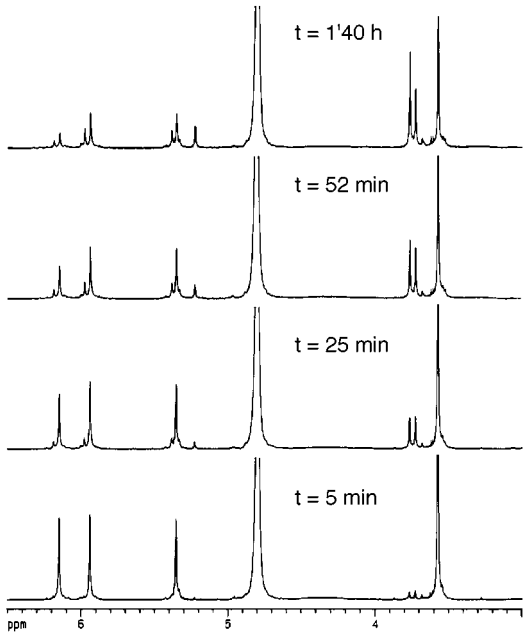


Figure 1. Racemization of **17a** followed by ^{1}H NMR (30 mg of **17a**, 10 μL of piperidine in 1 mL of $CDCl_3/D_2O$). The signals of the racemizing H_α at 6.18 and 6.15 ppm decrease due to interchange with deuterium.

chiral centers were present, we decided to study this reaction in more detail.

To this end, **17a** was dissolved in 1% piperidine in CD_3OD with a drop of D_2O in an NMR tube and the reaction followed by NMR. We expected to observe one of the H_α-singlets interchanging with deuterium and therefore disappearing. Unfortunately, under these conditions several reactions were observed, including hydrolysis of the ester bonds, and the spectra were too complex to be analyzed. When the same experiment was carried out in $CDCl_3$ with a drop of D_2O (Figure 1), two new compounds were the main products: one of these was methyl ester **8a**, which means that under the reaction conditions hydrolysis took place, and the other showed the expected second set of signals for the racemization product. However, most importantly, the intensities of the two singlets at 6.18 and 6.15 ppm, which correspond to the racemizing H_α, began to decrease slowly, as one would expect. At 1h 40 min, these signals had shrunk to about one-third of the intensity of the other H_α singlets, which belonged to the protons that were not affected by the base. By the same time, the ratio of the two products was 3:7. Consequently, in $CDCl_3/D_2O$ essentially the same reaction could be observed as in THF, although it was slower and complicated by the concurrent hydrolysis of the ester bonds.

To find out which one of the three centers underwent racemization, we synthesized the model compounds **20a**, **21a**, and **22a** (Scheme 4). The deprotection products **17a** and **18a** were methylated and compared with **20a**, **21a**, and **22a** by NMR and optical rotation. In this way, we determined that **18a** represented the "intact" deprotection product and that **17a** arose from racemization in the second mandelic acid residue.

In an attempt to suppress racemization, we carried out experiments with weaker bases such as morpholine, *N*-methylmorpholine, and imidazole (Table 2). Unfortunately, these modifications only served to prolong reaction times and required higher base concentrations, and they did not suppress racemization. When L-mandelic acid was replaced by L-α-hydroxyisovaleric acid (**4b**), tridepside **16b** could be obained by the same procedure as used for **16a** (Scheme 3). In this case, no racemization could be observed during deprotection of **16b**. Only one product, **17b**, was obtained.

Although Fmoc could probably be used for the solid-phase synthesis of depsides devoid of mandelic acid or other base-sensitive hydroxy acids, we decided to look for another protecting group because the risk of racemization limits the versatility of the methodology.

Finally, we elected to protect the hydroxyl group of the hydroxy acids as THP ethers and found it to be the protecting group of choice for depside synthesis. **7a** was obtained either from methyl ester **8a** by protection with dihydropyran/*p*-TsOH and subsequent hydrolysis of the methyl ester, by protection of the free acid **4a** with excess dihydropyran and hydrolysis of the THP ester, or also directly from **4a** with only a slight excess of dihydropyran. The best results were obtained with the last method: the yield (79%) is good and needs only one reaction step. Neither undesired deprotection nor racemization was observed during the coupling and deprotection steps leading to tridepside **17a** (Scheme 5). The major inconvenience of THP-protected chiral alcohols is the complexity of the NMR spectra due to formation of diastereoisomers, and this only interferes in the identification of those compounds that are prepared in solution (**7a**−**c**, **23a**, and **24a**). Since the THP-protected resin-bound intermediates do not have to be isolated and identified, this potential complication does not represent a problem in solid-phase synthesis.

Comparison of Coupling Systems. Following our preliminary studies in solution, DIC/DMAP was chosen for the depside couplings because it was found to be very efficient in esterification reactions carried out in our group.[24] Nevertheless, many more methods for the esterification of carboxylic acids are known. The growing popularity of uronium and phosphonium-salt based coupling reagents in peptide synthesis[25] led us to consider whether these reagents, alone or in combination with DMAP, might be useful for ester bond formation. Thus, a series of different coupling systems was tested to assess their utility in depside bond formation. The results are summarized in Table 3.

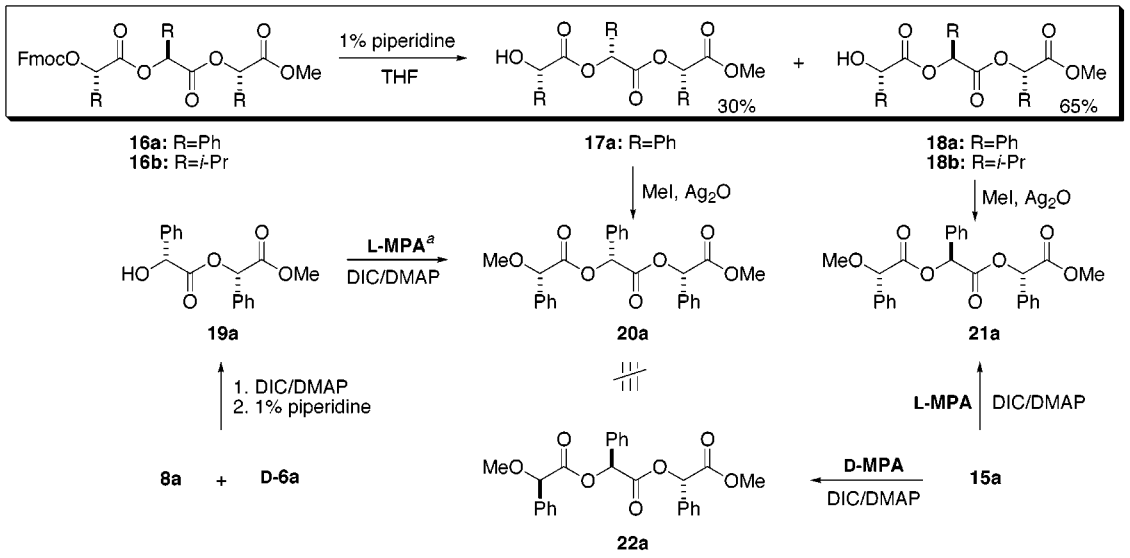
All the peptide coupling reagents that were tested (DIC, HBTU, HATU, PyBroP) gave poor results if no auxiliary reagent was used. Addition of HOBt in the case of DIC couplings helped to improve the yields but required rather long reaction times and the yields were strongly dependent on the water content of HOBt: when commercial (nondried) HOBt was used, which contains 1 equiv of water, hardly any product was isolated. Consequently, HOBt was not added for the other reagents tested.

HBTU, HATU, and PyBroP were virtually ineffective, either with or without DMAP, when 2,4,6-trimethylpyridine (collidine) was used in equimolar amounts in relation to the uronium salt, conditions that have been recommended to avoid racemization.[26] A 2-fold increase

(24) Latypov, S. K.; Seco, J. M.; Quiñoá, E.; Riguera, R. *J. Am. Chem. Soc.* **1998**, *120*, 877−882.

(25) Carpino, L. A.; El-Faham, A.; Minor, C. A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201−203, and references therein.

Scheme 4



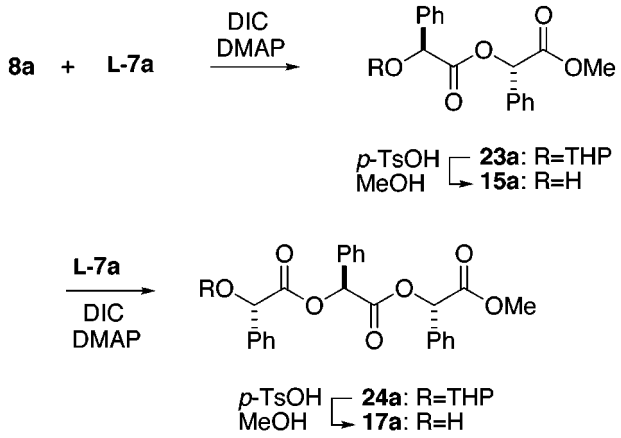
[a] MPA = α-methoxyphenylacetic acid.

Table 2. Racemization during Deprotection of 16a with Different Bases

base	pK_a[a]	t_{reac}	ratio[b]
piperidine 1−2%	11.12	40 min	65:35
morpholine 2%	8.33	4−5 h	86:14
4-Me-morpholine 8%	7.41	10 h	81:19
imidazole 10%	6.95	19 h	*c*

[a] Weast, R. C. *Handbook of Chemistry and Physics*, 56th ed.; CRC Press: Cleveland, OH, 1975; pp D147−149. [b] Ratio of **17a**:**18a** was determined by NMR (integration of the H_α signals in the region of 5.0−6.2 ppm). [c] More than two products.

Scheme 5



in the amount of base significantly improved the results, and good yields within reasonable reaction times could be obtained in the presence of DMAP. Nevertheless, none of the coupling methods led to the yields obtained with DIC/DMAP. In addition, the presence of 3 equiv of a base whose pK_a is similar to *N*-methylmorpholine (7.43, see footnote *a*, Table 2) represents an additional risk of racemization.

The best results were therefore obtained with DIC/DMAP. Studies with different reaction times have shown that 2 h is generally sufficient to obtain the maximum yield, and longer reaction times do not lead to increased yields. The yields obtained in solution with only a slight excees of O^α-protected hydroxy acid are good, and such reactions should almost reach completion on the resin when more equivalents of protected acid are employed.

Table 3. Reaction Times and Yields for Different Coupling Systems in Depside Bond Formation between 8a and 7a

coupling system[a,b]	solvent[c]	t_{reac} (h)	yield (%)
DIC/HOBt·H_2O[d]	A	2	28
	A	4	7
	A	20	2
DIC/HOBt[e]	A	2	19
	A	4	23
	A	20	64
DIC/DMAP[f]	A	0.5	79
	A	2	92
	A	4	90
HBTU[g]	A	15	16
HBTU[g]	B	4	5
HBTU/DMAP[f,h]	B	4	46
HATU[g]	A	4	5
HATU[g]	B	4	3
HATU/DMAP[f,g]	B	4	18
HATU/DMAP[f,h]	B	4	49
PyBroP[h]	B	4	6
PyBroP/DMAP[f,h]	B	4	60

[a] Abbreviations used: Bpoc = 2-(4-biphenylyl)-2-propyloxycarbonyl; DIC = diisopropylcarbodiimide; DMAP = 4-(dimethylamino)pyridine; HATU = O-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HBTU = *O*-(7-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOBt = 1-hydroxybenzotriazol; PyBrop = bromotripyrrolidinophosphonium hexafluorophosphate. [a] Reaction conditions: **8a** (1 equiv, 0.36 M), **7a** (1.2 equiv, 0.43 M), coupling reagent (1.4 equiv, 0.43 M), rt. [b] A: THF. B: CH_2Cl_2/DMF (1:1). [c] 1.4 equiv of commercial HOBt·H_2O (not dried). [d] 1.4 equiv of dried HOBt (8 h at 130 °C). [e] 0.1 equiv of DMAP. [f] With 1.4 equiv of 2,4,6-trimethylpyridine. [g] With 2.8 equiv of 2,4,6-trimethylpyridine.

Solid-Phase Synthesis. Among the existing solid supports, Wang resin was chosen and it proved to meet our needs well. The first THP-protected hydroxy acid was linked to the resin using the same conditions and reagents employed for all the other couplings, and this significantly simplifies the whole automatic process. The following reagents, ratios and conditions were employed for the depside couplings: for each equivalent of resin-attached substrate, 3.0 equiv of THP-protected hydroxy

(26) Han, Y.; Albericio, F.; Barany, G. *J. Org. Chem.* **1997**, *62*, 4307−4312.

Scheme 6

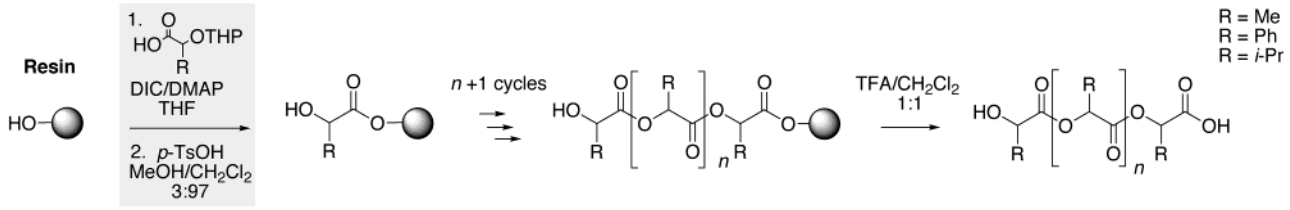
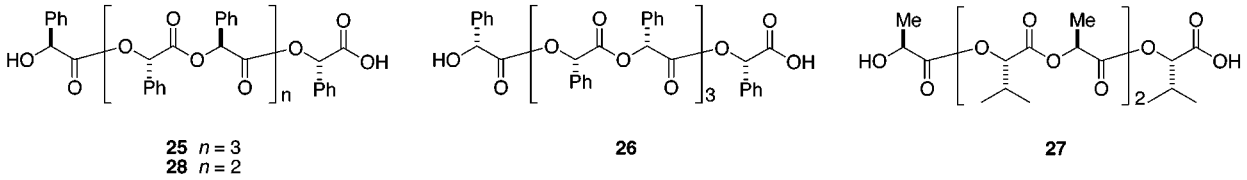


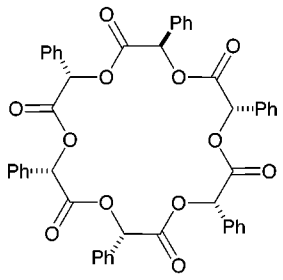
Chart 1



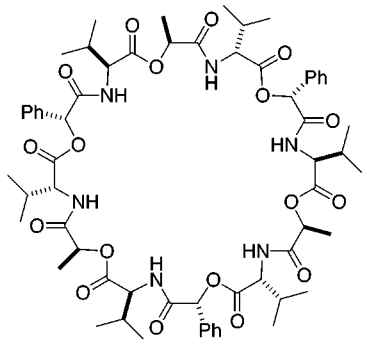
25 $n = 3$
28 $n = 2$

26

27



29



30

acid (0.3 mmol/mL), 3.0 equiv of DIC, and 0.1 equiv of DMAP for 2 h at room temperature in either THF or DMF.

While *p*-TsOH/MeOH was used to deprotect the THP group in solution,[27] it proved to be ineffective in the solid phase, probably due to poor swelling of the resin. Monitoring of the deprotection reactions by GC[28] revealed that introduction of CH_2Cl_2 as a cosolvent eliminated this problem, and a mixture of *p*-TsOH (5 mg/mL) in CH_2Cl_2/MeOH (97:3) was found to give the best results. With this solution, deprotection was complete after 45−70 min. Once the necessary minimum reaction time was known, and the absence of resin cleavage during this process was demonstrated by NMR of the concentrated washings, deprotections were carried out for 2 × 60 min. Final cleavage of the depside chain from the resin was accomplished with TFA/CH_2Cl_2 (1:1) and did not affect the depside bonds, as shown by the absence of depside fragments in the solution (checked by HPLC).

We have tried to find a simple procedure similar to the ninhydrin test for assessing the efficiency of coupling in depside bond formation. To this end, we developed an adaptation of a scarcely known TLC spray reagent for alcohols[29] that can be carried out directly on the resin and has shown to be a quick and reliable method for the detection of small amounts of resin-bound alcohol groups. The details of the procedure are described in the Experimental Section.

Scheme 6 summarizes, in a general way, the different steps of the solid-phase preparation of α-hydroxy acid-derived depsides. Several depside chains were prepared following this methodology. To test the versatility of the approach, different synthetic targets were chosen, which included the same enantiomer (i.e., H-[L-Man]$_8$-OH, **25**), both enantiomers (i.e., H-[D-Man-L-Man]$_4$-OH, **26**), or different hydroxy acids in the same chain (i.e., H-[L-Lac-L-Hiv]$_3$-OH, **27**).

Small resin samples were removed and cleaved at different stages of the syntheses of depsides **25**−**27** (Chart 1). In each case, the crude product mixture was purified by HPLC and the isolated depsides analyzed by NMR. Since the H_α protons of different diastereomers of the same depside, that may arise from racemization, show different signals in ^{1}H NMR, they can be easily distinguished and quantified. Thus, for all the synthetic intermediates and end products, the content of other diastereoisomers was established and found to be lower than 1%. Figure 2 shows the CD spectra of six depsides (from two to seven units) obtained from L-mandelic acid.

The overall yields for depsides **25**, **26**, and **27** lie between 72 and 75% (after HPLC isolation). This corresponds to an average value of 95−97% per cycle. The side products accounting for the remaining 3−5% per cycle were isolated by HPLC and identified as the (n + 1) oligomer (∼1%) and the depsides lacking one or more residues.

Cyclization of these open-chain depsides leads to a new

(27) Greene, T. W. *Protective Groups in Organic Synthesis*; Wiley: New York, 1981.

(28) Small samples of the deprotection solution were directly taken from the reaction vessel and injected onto a capillary column (Ultra-1, 25 m × 0.32 mm × 0.52 μm) at 60 °C. The peak area of the acetal resulting from cleavage of the THP group (t_R = 8.96 min) was compared to an internal standard (toluene, t_R = 5.89 min). Deprotection was complete when the ratio of these two areas was constant.

(29) Pomonis, J. G.; Severson, R. F.; Freeman, P. J. *J. Chromatogr.* **1969**, *40*, 78−84.

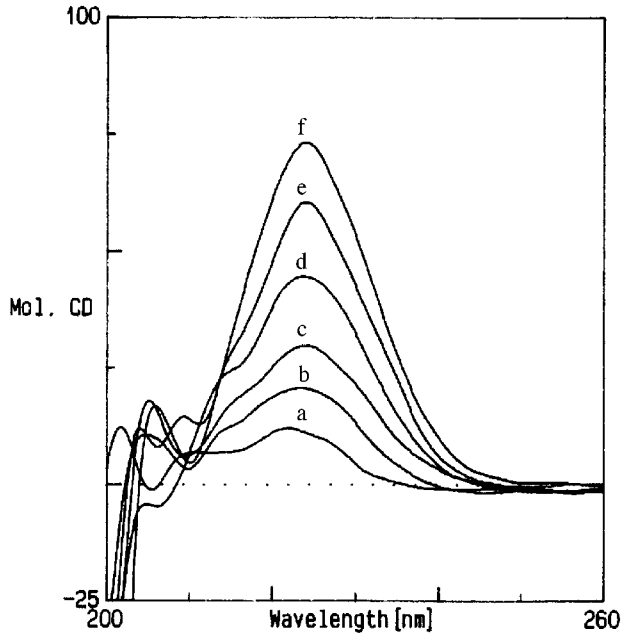


Figure 2. CD spectra of the intermediate depsides (from 2 to 7 units of L-mandelic acid) in the synthesis of **25**.

class of chiral macrocycles. In this way, when hexadepside **28** was made to undergo the Mitsunobu reaction[30] (diethyl azodicarboxylate, triphenylphosphine), cyclohexadepside **29**, a chiral ester analogue of 18-crown-6, was obtained.

To show the reliability of the method for the preparation of depsipeptides, total synthesis of the valinomycin analogue **30** ([L-Val-D-Man-D-Val-L-Lac]$_3$) was undertaken. The corresponding linear precursor was entirely synthesized on solid support as outlined in Scheme 7 and cyclized in solution. In this derivative, D-α-hydroxyisovaleric acid is replaced by D-mandelic acid, thus obtaining a new analogue while maintaining the favorable conformation of the authentic valinomycin. This represents a challenging target because of its six depside and peptide bonds. Bpoc (2-(4-biphenylyl)-2-propyloxycarbonyl)[31] was chosen as the protecting group for the α-amino groups because the deprotecting conditions (0.5% TFA/CH_2Cl_2, 20 min) are mild enough to be compatible with the Wang resin,[32] which in turn was selected on the basis of the relatively mild final cleavage conditions.

The initial idea was to use the pentafluorophenol ester protocol for the final cyclization, in combination with in situ release of the amino functionality by hydrogenolysis of a benzyloxycarbonyl group. These conditions have been shown to minimize polymerization and gave very good results in the synthesis of valinomycin analogues.[33] Thus, the Cbz-protected (Cbz = benzyloxycarbonyl) linear precursor **31** was synthesized, in 63% overall yield (Scheme 7), and then converted into the pentafluorophenol ester. Unfortunately, despite our efforts, we were not able to selectively cleave the Cbz group without damaging the substrate, which also bears benzyl moieties in its mandelic acid units. Therefore, depsipeptide **32** was prepared by the same procedure (Scheme 7) with the only difference being that Bpoc-L-valine instead of Cbz-L-valine was used for the final coupling step. Final cleavage of the product from the resin simultaneously cleaves the Bpoc group to give the unprotected linear depsipeptide **32** in 47% overall yield. The lower yield for **32**, compared to that obtained for **31**, is explained by the more difficult HPLC purification.

As far as the use of the Bpoc group for solid-phase preparation of depsipeptides is concerned, we found it to be more difficult to cleave than expected. In fact, when a resin sample obtained after four steps was cleaved and purified by HPLC, two main products were observed and could be identified by NMR as the tridepsipeptides H-D-Man-D-Val-L-Lac-OH and H-L-Val-D-Val-L-Lac-OH. Since all the depside/peptide coupling reactions had been monitored by the test described before and the Kaiser test and found to be complete, the problem must have arisen from incomplete deprotection. Indeed, we found that the Bpoc deprotection of resin-bound Bpoc-D-Val-L-Lac had not gone to completion in 20 min as noted in the literature.[34] Monitoring of Bpoc deprotection by analysis of the washings indicated that at least a 1 h treatment with 0.5% TFA was necessary to ensure complete deprotection. This does not represent a problem for the preparation of medium-sized molecules, but it can complicate the synthesis of larger chains because repeated deprotections of 1 h duration will cause a certain degree of cleavage of the anchoring bond,[32] thus forcing a change of linker or of protective groups.

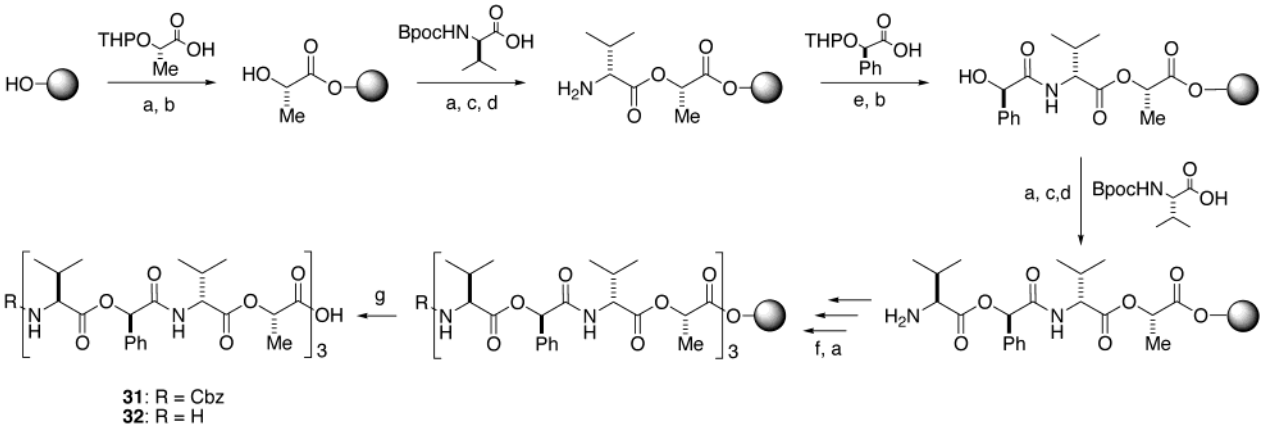
Macrolactamization of **32** was accomplished by two different procedures: via the acid chloride method[35] and with HATU,[36] thus obtaining **30** in 14% and 24% yield, respectively. In the case of the acid chloride method, we suspect that the acid chloride hydrochloride of **32** reacts spontaneously in the syringe, before addition of base. This would explain the presence of relatively large amounts of oligomerization products that were isolated in spite the high dilution. **30** represents a novel valinomycin derivative, and its bioactivity is currently under investigation.

In conclusion, we have developed a general methodology for the solid-phase synthesis of depsides and depsipeptides. The method gives high yields, ensures the stereochemical integrity of the products, and therefore, allows the preparation of relatively large substrates. The versatility of the method has been demonstrated by the preparation of different hydroxy and amino acid-containing substrates. Cyclization of the linear depsides leads

(30) Mitsunobu, O. *Synthesis* **1981**, 1−80.
(31) Sieber, P.; Iselin, B. *Helv. Chim. Acta* **1968**, *51*, 622−632.
(32) Wang, S. S. *J. Org. Chem.* **1975**, *40*, 1235−1239.
(33) For synthesis of valinomycin analogues in solution, see: Dory, Y. L.; Mellor, J. M.; McAleer, J. F. *Tetrahedron* **1996**, *52*, 1343−1360, 1361−1378, and 1379−1388.

(34) The CH_2Cl_2 washings from the deprotection steps, which had been collected separately, were concentrated and analyzed by ^{1}H NMR spectroscopy. The washings from the deprotection of the resin-bound tridepsipeptide THP-D-Man-D-Val-L-Lac gave a white solid instead of the usual brownish oil that results from cleavage of the THP group. The ^{1}H NMR spectrum of this solid was compared to that obtained from the Bpoc protection and showed the same signals. This means that Bpoc deprotection of the resin-bound didepsipeptide Bpoc-D-Val-L-Lac had not gone to completion and was accomplished in the subsequent THP cleavage with *p*-TsOH/MeOH.
(35) Losse, G.; Klengel, H. *Tetrahedron* **1971**, *27*, 1423−1434, and references therein.
(36) Jou, G.; González, I.; Albericio, F.; Lloyd-Williams, P.; Giralt, E. *J. Org. Chem.* **1997**, *62*, 354−366.

Scheme 7[a]



[a] Key: (a) DIC 3 equiv, DMAP 0.1 equiv, THF, 2 h; (b) *p*-TsOH (5 mg/mL), MeOH/CH_2Cl_2 (3:97), 2 h; (c) TFA/CH_2Cl_2 (0.5:99.5), 20 min; (d) (*i*-Pr)$_2$EtN/CH_2Cl_2 (5:95), 2 × 5 min; (e) DIC, THF, 2 h; (f) **31**: Cbz-L-Val; **32**: Bpoc-L-Val; (g) TFA/CH_2Cl_2 (1:1), 1 h.

to new class of chiral ionophores. The synthesis of **31** and **32** is, to our knowledge, the first entire solid-phase preparation (including formation of depside bonds on resin) of a depsipeptide that contains six depside bonds.

Experimental Section

General Methods. Unless specified, reagents were purchased from Aldrich or Fluka and were used without further purification. CH_2Cl_2 and THF used for solid-phase reactions were synthesis grade, and acetone and MeCN were HPLC grade. Solvents used for solution reactions were dried in the appropriate manner[37] and distilled prior to use. Wang resin was purchased from Advanced Chem Tech (loading capacity 1.0 mmol/g). Solid-phase reactions were carried out on an ACT90 peptide synthesizer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker DPX-250, AMX-300, and AMX-500 spectrometers. Coupling constants (*J*) are reported in Hz. The matrix for mass spectrometry was either thioglycerol or *m*-NBA. Normal-phase HPLC was carried out using a μ-Porasil (10 μ, 7.8 · 300 mm) column, reversed-phase HPLC with Nucleosil C_{18} (5 μ, 7.0 · 300 mm), or Partisil M9 ODS-3 (10 μ, 9.4 · 500 mm) columns. For all solid-phase reactions, yields are referred to purified material and based upon the loading of the starting resin.

General Procedure for Cleavage of Fmoc-Protected Depsides in Solution. A solution of the Fmoc-protected depside in piperidine/THF (1:99, 20 mL/mmol depside) was stirred at room temperature and the reaction followed by TLC. When the starting material had completely disappeared (30−40 min), the solution was concentrated to about 1 mL, dissolved in CH_2Cl_2, washed with 1 N HCl and water, dried (Na_2SO_4), filtered, and concentrated.

General Procedures for Solid-Phase Reactions. A. Depside coupling conditions: DIC (3 equiv), DMAP (0.1 equiv), THP-protected α-hydroxy acid or Bpoc-protected α-amino acid (3 equiv), in THF (1 mL/100 mg of resin), 2 h; washings (3 min each): 3 × 10 mL of THF; 3 ×10 mL of acetone; 3 × 10 mL of CH_2Cl_2.

B. Peptide coupling conditions: DIC (3 equiv), THP-protected α-hydroxy acid (3 equiv), in THF (1 mL/100 mg of resin), 2 h; washings (3 min each): 3 ×10 mL of THF; 3 ×10 mL of acetone; 3 ×10 mL of CH_2Cl_2.

C. THP deprotection: 10 mL of a solution of *p*-TsOH (5 mg/mL) in CH_2Cl_2/MeOH (97:3), 2 × 1 h, preceded by a 3 min washing with the same solution; washings (3 min each): 3 × 10 mL of CH_2Cl_2; 3 × 10 mL of acetone; 3 × 10 mL of THF.

D. Bpoc deprotection: 0.5% TFA in CH_2Cl_2 (10 mL), 20 min; 2 × 10 mL of CH_2Cl_2, 2 min each; 2 × 10 mL of 5% DIEA in CH_2Cl_2, 5 min; washings (3 min each): 3 × 10 mL of CH_2Cl_2; 3 × 10 mL of acetone; 3 × 10 mL of THF.

Experimental Procedure for the Solid-Phase Test of Alcohols. After each coupling step, a suspension of a small sample (A) of resin beads (≃1−2 mg dry resin weight) in a few drops of CH_2Cl_2 was placed on a silica gel TLC plate and spread out forming a thin circle. On the same plate, two "reference spots" were placed by the same procedure: sample B formed by OH-bearing resin (completely deprotected depside-linked resin) and sample C formed by fully acetylated Wang resin. Next, the spots were successively treated with the following reagent systems: first, 1−2 drops of 0.01 M TsCl in toluene; second, 1−2 drops of 0.025 M PNBP in toluene, then the TLC plate was heated with a heat gun until the orange color disappeared; third, 10% piperidine in $CHCl_3$ and heated until sample B showed a deep violet color whereas sample C remained colorless. If the coupling had been complete (no free OH present), sample A should have appeared colorless (negative test) as sample C. If color appeared, coupling had been incomplete and needed to be repeated. We observed a negative test when the resin contained less than 1% free OH groups.

***O*$^\alpha$-(*tert*-Butyldimethylsilyl)-L-mandelic Acid (L-5a).** To a solution of L-mandelic acid (152 mg, 1 mmol) and *tert*-butyldimethylsilyl chloride (331 mg, 2.2 mmol) in THF (3 mL) was added imidazole (163 mg, 2.4 mmol) at 4 °C, and the mixture was stirred for 1 h. The ice bath was removed, and the mixture was stirred for a further 3 h at room temperature. The reaction mixture was filtered, concentrated under reduced pressure, and suspended in 1 N NaOH (3 mL). After 1.75 h, the mixture was diluted with 3 mL of water, washed twice with ether, acidified with concentrated HCl to pH 3−4, and extracted three times with ether. The combined ether extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give L-**5a** (237 mg, 89%) as a sticky, colorless oil: $[\alpha]_D = +89.4$ ($c = 1.3$, $CHCl_3$); IR (NaCl) ν_{max} 3064, 3033, 2933, 2859, 1725, 1463, 1256, 1128, 866, 841, 781, 717, 699; ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.05 (s, 3 H), 0.15 (s, 3 H), 0.96 (s, 9 H), 5.26 (s, 1 H), 7.34−7.52 (m, 5 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ −5.3, −5.1, 18.1, 25.6, 74.2, 126.5, 128.5 (2×), 138.2, 175.9; FABMS *m*/*z* 289 $[M + Na]^+$ (13), 267 $[M + H]^+$ (9), 249 (30), 221 (100). Anal. Calcd for $C_{14}H_{22}SiO_3$: C, 63.12; H, 8.32. Found: C, 62.85; H, 8.09.

***O*$^\alpha$-(9-Fluorenylmethyloxycarbonyl)mandelic Acid (6a)**. A solution of pyridine (423 μL, 5.25 mmol) in THF (2 mL) was added during 20 min to a solution of mandelic acid (L or d, 761 mg, 5 mmol) and 9-fluorenylmethyloxycarbonyl chloride (1358 mg, 5.25 mmol) in THF (13 mL) at 4 °C. The mixture was stirred for 2 h at 4 °C and then allowed to warm to room temperature. After 15 h, the mixture was filtered and concentrated under reduced pressure. The crude product was dissolved in CH_2Cl_2 and washed with 1 N HCl and water. After drying (Na_2SO_4), filtering, and concentration under reduced

(37) Perrin, D.; Armaregeo, W. L. F. *Purification of Laboratory Chemicals*; Pergamon Press: Oxford, 1988.

pressure, the product was crystallized from $CHCl_3$ to give **6a** (1084 mg, 58%) as a white solid: mp = 175 °C (toluene); $[\alpha]_D$ = +94.9 (c = 1.2, $CHCl_3$); IR (NaCl) ν_{max} 3400 br, 3039, 1741, 1590, 1448, 1390, 1252, 951, 736 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 4.30 (t, J = 7.3, 1 H), 4.40 (dd, J_1 = 10.2, J_2 = 7.6, 1 H), 4.54 (dd, J_1 = 10.2, J_2 = 7.2, 1 H), 5.92 (s, 1 H), 7.27−7.44 (m, 7 H), 7.52−7.56 (m, 2 H), 7.64 (dd, J_1 = 7.5, J_2 = 0.92, 1 H), 7.77 (d, J = 7.5, 2 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 46.6, 70.7, 76.8, 120.1, 125.2, 125.3, 127.3, 127.7, 128.0, 129.0, 129.8, 132.7, 141.4, 143.1, 143.3, 154.5, 173.5; EIMS m/z 374 $[M]^+$ (2), 178 (100), 165 (40); HREIMS m/z 374.1159 (calcd for $C_{23}H_{18}O_5$ $[M]^+$ 374.1154). Anal. Calcd for $C_{23}H_{18}O_5$: C, 73.77; H, 4.85. Found: C, 73.96; H, 4.62.

***O*$^\alpha$-(9-Fluorenylmethyloxycarbonyl)-L-α-hydroxyisovaleric Acid (L-6b).** L-**6b** was prepared as described above for L-**6a** from L-α-hydroxyisovaleric acid (236 mg, 2 mmol). Crystallization from toluene gave L-**6b** (259 mg, 54%) as a white solid: $[\alpha]_D$ = +2.9 (c = 2.8, $CHCl_3$); IR (NaCl) ν_{max} 2971, 1749, 1452, 1391, 1256, 1136, 1110, 992, 742, 674, 625 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.11 (t, J = 7.16, 6 H), 2.36 (m, 1 H), 4.31−4.40 (m, 2 H), 4.53 (dd, J_1 = 9.4, J_2 = 6.4, 1 H), 4.88 (d, J = 4.1, 1 H), 7.33 (dt, J_1 = 7.5, J_2 = 1.1, 2 H), 7.42 (t, J = 7.5, 2 H), 7.65 (t, J = 7.9, 2 H), 7.77 (d, J = 7.2, 2 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 17.0, 18.5, 30.2, 46.8, 70.4, 79.5, 120.1, 125.2, 125.3, 127.2, 127.3, 127.9, 141.4, 143.2, 143.5, 155.0, 173.5; EIMS m/z 340 (3), 178 (100). Anal. Calcd for $C_{20}H_{20}O_5$: C, 70.57; H, 5.92. Found: C, 70.20; H, 5.89.

***O*$^\alpha$-Tetrahydropyranyl-L-mandelic Acid (L-7a).** Dihydropyran (2.55 mL, 28 mmol) was added dropwise by syringe to a stirred suspension of L-mandelic acid (3043 mg, 20 mmol) and p-TsOH (76 mg, 0.4 mmol) in $CHCl_3$ (40 mL) at 4 °C. After 5 min, the ice bath was removed and the mixture allowed to warm to room temperature. After 15−30 min, all of the compounds had completely dissolved and a violet color began to appear. After 1.5 h, the reaction mixture was extracted with 0.2 N KOH (2 · 50 mL). The combined KOH layers were acidified with 6 N HCl to pH 3−4 and extracted three times with CH_2Cl_2. The pH of the aqueous layer was controlled between extractions, with more HCl added if necessary. The combined CH_2Cl_2 extracts were washed with water, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give L-**7a** (3734 mg, 79%) as a sticky oil that solidified slowly to give a colorless, waxy solid: IR (NaCl) ν_{max} 2944, 2870, 1731, 1494, 1448, 1390, 1266, 1183, 1124, 1071, 1029, 977, 907, 865, 704 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.44−1.94 (m, 6 H), 3.50−3.58 (m, 1 H), 3.61−3.76 and 3.91−4.01 (m, 1 H), 4.60 (t, J = 3.5) and 4.93 (m, 1 H), 5.25 and 5.33 (s, 1 H), 7.33−7.52 (s, 5 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 18.5, 19.0, 25.0, 25.1, 30.0, 30.1, 61.9, 62.6, 75.4, 76.4, 96.7, 97.2, 127.2, 127.5, 128.5, 128.6, 128.8, 135.6, 136.1, 174.8, 176.2; FABMS m/z 259 $[M + Na]^+$ (39), 237 $[M + H]^+$ (50); HRFABMS m/z 237.1128 (calcd for $C_{13}H_{17}O_4$ $[M + H]^+$ 237.1127). Anal. Calcd for $C_{13}H_{16}O_4$: C, 66.07; H, 6.83. Found: C, 66.23; H, 6.71.

***O*$^\alpha$-Tetrahydropyranyl-L-α-hydroxyisovaleric Acid (L-7b).** L-**7b** was prepared as described above for L-**7a** from L-α-hydroxyisovaleric acid (2364 mg, 20 mmol). For the extraction of the acidified KOH layers, $CHCl_3$/i-PrOH (3:1) was used instead of CH_2Cl_2 and the aqueous phase saturated with NaCl before the extraction. L-**7b** (2987 mg, 74%) was obtained as a sticky oil: IR (NaCl) ν_{max} 3600−3400, 2956, 2871, 1725, 1466, 1389, 1204, 1130, 1030, 973, 910, 865, 806 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 0.91−1.04 (m, 6 H), 1.45−1.85 (m, 6 H), 2.07−2.20 (m, 1 H), 3.44−3.55 (m, 1 H), 3.80−4.05 (m, 1 H), 3.80 (d, J = 5.0) and 4.11 (d, J = 4.9, 1 H), 4.52−4.55 and 4.68−4.70 (m, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 17.2, 17.4, 18.5, 18.8, 19.2, 19.9, 24.9, 25.3, 30.2, 30.4, 31.2, 31.3, 62.1, 63.9, 78.3, 83.4, 96.9, 102.1, 176.1, 177.5; FABMS m/z 225 $[M + Na]^+$ (100), 203 $[M + H]^+$ (31). Anal. Calcd for $C_{10}H_{18}O_4$: C, 59.37; H, 8.98. Found: C, 59.41; H, 9.05.

***O*$^\alpha$-Tetrahydropyranyl-L-lactic acid (L-7c).** L-**7c** was prepared, as described above for L-**7b**, from L-lactic acid (1816 mg, 20 mmol) and was obtained as an oil (2471 mg, 71%): IR (NaCl) ν_{max} 3500 br, 2944, 2873, 1729, 1456, 1376, 1213, 1129, 1025, 984, 897, 866, 815 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.40−1.87 (m, 6 H), 1.43 (d, J = 6.9) and 1.48 (d, J = 7.1, 3 H), 3.48−3.57 (m, 1 H), 3.80−3.89 (m) and 3.92−3.99 (m, 1 H), 4.25 (q, J = 6.9) and 4.43 (q, J = 7.0, 1 H), 4.65−4.68 and 4.72−4.74 (m, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 17.8, 18.6, 19.1, 19.9, 24.9, 25.2, 30.3, 30.7, 62.5, 64.0, 70.0, 73.2, 97.9, 99.9, 176.1, 178.2; HREIMS m/z 174.0891 (calcd for $C_8H_{14}O_4$ $[M]^+$ 174.0892). Anal. Calcd for $C_8H_{14}O_4$: C, 55.14; H, 8.10. Found: C, 54.96; H, 8.22.

L-Mandelic Acid Methyl Ester (8a). To a stirred solution of L-mandelic acid (3043 mg, 20 mmol) in MeOH (6 mL) was added thionyl chloride (1.6 mL, 22 mmol) dropwise at 4 °C. Then the solution was brought to reflux. After 4 h, the mixture was concentrated under reduced pressure, dissolved in CH_2Cl_2, extracted with a saturated solution of $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated under reduced pressure to give **8a** (2988 mg, 90%) as a slightly yellow solid: ^1H NMR (250 MHz, $CDCl_3$) δ 3.74 (s, 3 H), 5.19 (s, 1 H), 7.28−7.48 (m, 5 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 53.0, 74.8, 126.5, 128.4, 128.5, 138.2, 174.0.

Depside Coupling between L-5a and L-8a. A solution of L-**8a** (50 mg, 0.30 mmol), L-**5a** (96 mg, 0.36 mmol), DIC (66 μL, 0.42 mmol), and DMAP (4 mg, 0.03 mmol) in THF (1 mL) was stirred at room temperature for 2 h. The reaction mixture was filtered and concentrated under reduced pressure. Purification by flash chromatography with hexane/EtOAc (7:3) gave 93 mg of a complex product mixture. This mixture was separated by HPLC (μ-Porasil, hexane/EtOAc (91:9), 2.0 mL/min) to give **9** (34 mg, 38%), **10** (22 mg, 17%), and **11** (7 mg, 4%). ***O*$^\alpha$-(*tert*-Butyldimethylsilyl)-L-mandelyl-L-mandelic acid methyl ester (9):** $[\alpha]_D$ = +89.1 (c = 0.9, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 0.04 (s, 3 H), 0.13 (s, 3 H), 0.92 (s, 9 H), 3.59 (s, 3 H), 5.36 (s, 1 H), 5.91 (m, 1 H), 7.33−7.56 (m, 10 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ −5.2, −5.1, 18.3, 25.7, 52.4, 74.2, 74.9, 126.6, 127.5, 128.2, 128.7, 129.2, 133.6, 138.6, 168.6, 171.4; HRFABMS m/z 415.1939 (calcd for $C_{23}H_{31}O_5Si$ $[M + H]^+$ 415.1941). ***O*$^\alpha$-(*tert*-Butyldimethylsilyl)-L-mandelyl-L-mandelyl-L-mandelic acid methyl ester (10):** $[\alpha]_D$ = +89.3 (c = 1.2, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 0.02 (s, 3 H), 0.11 (s, 3 H), 0.91 (s, 9 H), 3.54 (s, 3 H), 5.37 (s, 1 H), 5.92 (s, 1 H), 6.06 (s, 1 H), 7.29−7.55 (s, 15 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 18.3, 25.6, 52.4, 74.2, 74.6, 75.1, 126.6, 127.5, 127.9, 128.2, 128.3, 128.6, 128.7, 129.3, 133.2, 138.6, 167.4, 168.3, 171.2; HRFABMS m/z 549.2307 (calcd for $C_{31}H_{37}O_7Si$ $[M + H]^+$ 549.2309). ***O*$^\alpha$-(*tert*-Butyldimethylsilyl)-L-mandelyl-L-mandelyl-L-mandelyl-L-mandelic acid methyl ester (12):** ^1H NMR (250 MHz, $CDCl_3$) δ 0.00 (s, 3 H), 0.08 (s, 3 H), 0.88 (s, 9 H), 3.51 (s, 3 H), 5.35 (s, 1 H), 5.87 (s, 1 H), 6.05 (s, 1 H), 6.06 (s, 1 H), 7.25−7.58 (s, 20 H).

***O*$^\alpha$-(9-Fluorenylmethyloxycarbonyl)-L-mandelyl-L-mandelic Acid Methyl Ester (14a).** A solution of L-**8a** (50 mg, 0.30 mmol), L-**5a** (135 mg, 0.36 mmol), DIC (66 μL, 0.42 mmol), and DMAP (4 mg, 0.03 mmol) in THF (1 mL) was stirred at room temperature for 2 h. The reaction mixture was filtered and concentrated under reduced pressure. Purification by flash chromatography with hexane/EtOAc (7:3) gave **14a** (138 mg, 88%) as an amorphous solid: $[\alpha]_D$ = +78.1 (c = 3.1, $CHCl_3$); IR (NaCl) ν_{max} 3063, 3039, 2960, 1753, 1495, 1448, 1385, 1255, 1171, 1033, 966, 912, 738, 699 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 3.61 (s, 3 H), 4.31−4.44 (m, 2 H), 4.57 (dd, J_1 = 9.6, J_2 = 6.6, 1 H), 6.04 (s, 1 H), 6.10 (s, 1 H), 7.29−7.59 (m, 12 H), 7.63−7.69 (m, 4 H), 7.79 (d, J = 7.3, 2 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 46.3, 52.4, 70.4, 74.9, 77.2, 119.8, 125.0, 125.1, 127.0, 127.7, 127.8, 128.5, 128.7, 129.0, 129.4, 132.6, 133.0, 141.0, 142.9, 143.2, 154.2, 167.5, 168.2; FABMS m/z 522 $[M]^+$ (5), 477 (17), 329 (17), 178 (100); HRFABMS m/z 522.1678 (calcd for $C_{32}H_{26}O_7$ $[M]^+$ 522.1679). Anal. Calcd for $C_{32}H_{26}O_7$: C, 73.54; H, 5.02. Found: C, 73.73; H, 5.03.

***O*$^\alpha$-(9-Fluorenylmethyloxycarbonyl)-L-α-hydroxyisovaleryl-L-α-hydroxyisovaleric Acid Methyl Ester (14b).** A solution of L-**8b** (53 mg, 0.40 mmol), L-**5b** (163 mg, 0.48 mmol), DIC (88 μL, 0.56 mmol), and DMAP (5 mg, 0.04 mmol) in THF (1 mL) was stirred at room temperature for 2 h. The reaction mixture was filtered and concentrated under reduced pressure. Purification by flash chromatography with hexane/EtOAc (4:1) gave **14b** (162 mg, 90%) as an amorphous solid: $[\alpha]_D$ = −27.8 (c = 1.2, $CHCl_3$); IR (NaCl) ν_{max} 2967, 2883, 1751,

1451, 1387, 1289, 1256, 1197, 1132, 1024, 1001, 792, 741 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.01 (dd, $J_1 = 9.3$, $J_2 = 6.9$, 6 H), 1.15 (dd, $J_1 = 6.9$, $J_2 = 4.2$, 6 H), 2.23−2.45 (m, 2 H), 3.74 (s, 3 H), 4.28−4.38 (m, 2 H), 4.44−4.51 (m, 1 H), 4.92 (d, $J =$ 4.2, 1 H), 4.97 (d, $J = 4.3$, 1 H), 7.33 (t, $J = 7.4$, 2 H), 7.42 (t, $J = 7.3$, 2 H), 7.65 (t, $J = 8.2$, 2 H), 7.77 (d, $J = 7.4$, 2 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 16.9, 17.1, 18.5, 30.1, 30.3, 46.6, 52.0, 70.3, 70.3, 79.6, 120.0, 125.2, 125.3, 125.8, 127.1, 127.2, 127.8, 141.2, 143.1, 143.5, 154.9, 169.0, 169.5; FABMS *m/z* 455 $[M + H]^+$ (11), 277 (11), 245 (12), 178 (100). Anal. Calcd for $C_{26}H_{30}O_7$: C, 68.71; H, 6.65. Found: C, 68.97; H, 6.78.

L-Mandelyl-L-mandelic Acid Methyl Ester (15a). (a) By Deprotection of 14a. 14a (138 mg, 0.26 mmol) was treated with piperidine/THF according to the general procedure. Flash chromatography of the crude product with hexane/EtOAc (7:3) gave **15a** (75 mg, 94%) as a pale yellow solid. (**b**) **By Deprotection of 23a. 23a** was treated with 6 mL of a solution of *p*-TsOH in MeOH (1 mg/mL), and the reaction was followed by TLC. After 1.5 h, the mixture was concentrated under reduced pressure to 1 mL, dissolved in ether, washed with $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. Purification of the crude product by flash chromatography with hexane/EtOAc (4:1) gave **15a** (152 mg, 91%) as a pale yellow solid: mp = 74−75 °C; $[\alpha]_D = +117.2$ ($c = 7.0$, $CHCl_3$); IR (NaCl) ν_{max} 3480 br, 3063, 3034, 2953, 1749, 1447, 1273, 1217, 1174, 1092, 1068, 1031, 736, 698 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 3.55 (d or s, $J = 5.4$ Hz, 1 H), 3.59 (s, 3 H), 5.33 (d or s, $J = 5.4$ Hz, 1 H), 6.01 (s, 1 H), 7.35−7.59 (m, 10 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 52.4, 73.1, 75.6, 126.9, 127.6, 128.5, 128.6, 128.9, 129.5, 133.3, 137.8, 168.3, 172.8; FABMS *m/z* 301 $[M + H]^+$ (37), 283 (26), 149 (88), 133 (90), 121 (100); HRFABMS *m/z* 301.1077 (calcd for $C_{17}H_{17}O_5$ $[M + H]^+$ 301.1076). Anal. Calcd for $C_{17}H_{16}O_5$: C, 67.98; H, 5.37. Found: C, 67.84; H, 5.46.

L-α-Hydroxyisovaleryl-L-α-hydroxyisovaleric Acid Methyl Ester (15b). 14b (162 mg, 0.36 mmol) was treated with piperidine/THF according to the general procedure. Flash chromatography of the crude product with hexane/EtOAc (4:1) gave **15b** (77 mg, 92%) as an amorphous solid: $[\alpha]_D = -13.6$ ($c = 2.0$, $CHCl_3$); IR (NaCl) ν_{max} 3500 br, 2966, 2882, 1745, 1463, 1370, 1280, 1208, 1135, 1029, 774 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 0.94−1.08 (m, 12 H), 2.14−2.30 (m, 3 H), 2.68 (d, $J = 6.2$, 1 H), 3.74 (s, 3 H), 4.12 (dd, $J_1 = 5.9$, $J_2 = 3.5$, 1 H), 4.91 (d, $J = 4.3$, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 15.7, 17.1, 18.6, 30.1, 32.3, 52.1, 74.8, 77.6, 169.5, 174.7; FABMS *m/z* 255 $[M + Na]^+$ (41), 233 $[M + H]^+$ (60), 179 (100), HRFABMS *m/z* 233.1388 (calcd for $C_{11}H_{21}O_5$ $[M + H]^+$ 233.1389). Anal. Calcd for $C_{11}H_{20}O_5$: C, 56.88; H, 8.68. Found: C, 56.96; H, 8.71.

***O*$^{\alpha}$-(9-Fluorenylmethyloxycarbonyl)-L-mandelyl-L-mandelyl-L-mandelic Acid Methyl Ester (16a).** A solution of **15a** (75 mg, 0.25 mmol), L-**5a** (85 mg, 0.36 mmol), DIC (66 μL, 0.42 mmol), and DMAP (4 mg, 0.03 mmol) in THF (1 mL) was stirred at room temperature for 2 h. The reaction mixture was filtered and concentrated under reduced pressure. Purification by flash chromatography with hexane/EtOAc (7:3) gave **16a** (147 mg, 90%) as an amorphous solid: $[\alpha]_D = +81.6$ ($c = 3.0$, $CHCl_3$); IR (NaCl) ν_{max} 3064, 3037, 2953, 1753, 1449, 1256, 1210, 1166, 1031, 737, 696 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 3.54 (s, 3 H), 4.28−4.41 (m, 2 H), 4.54 (dd, $J_1 = 9.4$, $J_2 =$ 6.4, 1 H), 5.93 (s, 1 H), 6.09 (s, 1 H), 6.16 (s, 1 H), 7.30−7.51 (m, 17 H), 7.60−7.66 (m, 4 H), 7.78 (d, $J = 7.4$, 2 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 46.6, 52.4, 70.7, 75.1, 75.2, 77.2, 120.0, 125.3, 125.4, 127.3, 127.5, 127.8, 127.9, 128.1, 128.8, 128.9, 129.3, 129.5, 129.7, 132.7, 133.1, 141.3, 143.1, 143.7, 154.5, 167.1, 167.8, 168.2; FABMS *m/z* 657 $[M + H]^+$ (100), 611 (76), 463 (60); HRFABMS *m/z* 657.2114 (calcd for $C_{40}H_{33}O_9$ $[M + H]^+$ 657.2124). Anal. Calcd for $C_{40}H_{32}O_9$: C, 73.15; H, 4.91. Found: C, 73.07; H, 4.94.

***O*$^{\alpha}$-(9-Fluorenylmethyloxycarbonyl)-L-α-hydroxyisovaleryl-L-α-hydroxyisovaleryl-L-α-hydroxyisovaleric Acid Methyl Ester (16b).** A solution of **15b** (77 mg, 0.33 mmol), L-**5b** (135 mg, 0.40 mmol), DIC (73 μL, 0.46 mmol), and DMAP (4 mg, 0.03 mmol) in THF (1 mL) was stirred at room temperature for 2 h. The reaction mixture was filtered and concentrated under reduced pressure. Purification of the residue by flash chromatography with hexane/EtOAc (4:1) gave **16b** (160 mg, 87%) as an amorphous solid: $[\alpha]_D = -32.0$ ($c =$ 1.4, $CHCl_3$); IR (NaCl) ν_{max} 3063, 2968, 2882, 1752, 1453, 1386, 1287, 1256, 1190, 1128, 1026, 789, 741 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.00 (dd, $J_1 = 9.2$, $J_2 = 6.9$, 6 H), 1.09 (dd, J_1 = 6.9, $J_2 = 4.5$, 6 H), 1.13 (dd, $J_1 = 6.9$, $J_2 = 4.4$, 6 H), 2.22−2.47 (m, 3 H), 3.72 (s, 3 H), 4.29−4.38 (m, 2 H), 4.43−4.52 (m, 1 H), 4.91 (d, $J = 4.3$, 1 H), 4.93 (d, $J = 4.5$, 1 H), 5.03 (d, $J =$ 4.1, 1 H), 7.32 (t, $J = 7.4$, 2 H), 7.41 (t, $J = 7.3$, 2 H), 7.64 (t, $J = 8.2$, 2 H), 7.77 (d, $J = 7.5$, 2 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 16.8, 16.9, 17.1, 18.5, 18.6, 30.1, 30.2, 30.3, 46.6, 52.0, 70.3, 77.2, 79.5, 120.0, 125.2, 125.4, 127.1, 127.2, 127.8, 141.2, 143.1, 143.5, 168.7, 169.1, 169.6; FABMS *m/z* 577 $[M + Na]^+$ (57), 555 $[M + H]^+$ (61), 377 (100), 345 (95); HRFABMS *m/z* 555.2592 (calcd for $C_{31}H_{39}O_9$ $[M + H]^+$ 555.2594). Anal. Calcd for $C_{31}H_{38}O_9$: C, 67.13; H, 6.91. Found: C, 67.28; H, 7.01.

Deprotection of 16a. 16a (147 mg, 0.22 mmol) was treated with piperidine/THF according to the general procedure. Flash chromatography of the crude product with hexane/EtOAc (7:3) gave 88 mg of a mixture. This mixture was separated by HPLC (μ-Porasil, hexane/EtOAc (8:2), 2.0 mL/min) to give 29 mg of **17a** ($t_R = 26$ h 10 min) and 54 mg of **18a** ($t_R = 29$ h 45 min). **l-Mandelyl-D-mandelyl-L-mandelic acid methyl ester (17a):.** $[\alpha]_D = +37.8$ ($c = 2.0$, $CHCl_3$); IR (NaCl) ν_{max} 3400 br, 3063, 3034, 2953, 2924, 2854, 1751, 1495, 1449, 1259, 1210, 1171, 1041, 737, 697 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 3.36 (s or d, $J = 5.7$, 1 H), 3.71 (s, 3 H), 5.36 (d, $J = 5.7$, 1 H), 5.98 (s, 1 H), 6.18 (s, 1 H), 7.27−7.44 (m, 15 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 52.7, 72.9, 75.1, 75.2, 126.7, 127.2, 127.3, 128.5, 128.6, 128.7, 129.4, 129.2, 132.6, 133.0, 137.5, 167.2, 168.2, 172.5; FABMS *m/z* 435 $[M + H]^+$ (8), 369 (9), 277 (13), 195 (88), 133 (100); HRFABMS *m/z* 435.1443 (calcd for $C_{25}H_{23}O_7$ $[M + H]^+$ 435.1444). **l-Mandelyl-L-mandelyl-L-mandelic acid methyl ester (18a):** $[\alpha]_D = +115.3$ ($c = 2.8$, $CHCl_3$); IR (NaCl) ν_{max} 3400 br, 2918, 2853, 1750, 1587, 1456, 1205, 1167, 1044, 792, 738, 699 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 3.41 (s or d, $J =$ 5.8, 1 H), 3.55 (s, 3 H), 5.33 (d, $J = 5.8$, 1 H), 5.93 (s, 1 H), 6.15 (s, 1 H), 7.27−7.57 (m, 15 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 52.4, 73.2, 75.2, 75.4, 126.9, 127.5, 127.9, 128.6, 128.8, 129.4, 129.6, 132.9, 133.2, 137.7, 167.2, 168.2, 172.6; FABMS *m/z* 435 $[M + H]^+$ (5), 369 (8), 277 (19), 185 (38), 133 (100); HRFABMS *m/z* 435.1443 (calcd for $C_{25}H_{23}O_7$ $[M + H]^+$ 435.1444). Anal. Calcd for $C_{25}H_{22}O_7$: C, 69.10; H, 5.11. Found: C, 69.05; H, 5.18.

18a by Deprotection of 24a. 18a was obtained, as described above for **15a**, by deprotection of **24a** with 5 mL of *p*-TsOH/MeOH to give an amorphous solid (170 mg, 87%).

L-α-Hydroxyisovaleryl-L-α-hydroxyisovaleryl-L-α-hydroxyisovaleric Acid Methyl Ester (18b). 16b (160 mg, 0.29 mmol) was treated with piperidine/THF according to the general procedure. Flash chromatography of the crude product with hexane/EtOAc (4:1) gave **18b** (87 mg, 91%) as an amorphous solid: $[\alpha]_D = -25.4$ ($c = 0.7$, $CHCl_3$); IR (NaCl) ν_{max} 3540 br, 2968, 2881, 1750, 1464, 1372, 1282, 1196, 1131, 1029, 777 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 0.94−1.09 (m, 18 H), 2.12−2.44 (m, 3 H), 2.65 (d, $J = 6.4$, 1 H), 3.73 (s, 3 H), 4.13 (dd, $J_1 = 6.0$, $J_2 = 3.4$, 1 H), 4.93 (d, $J = 4.3$, 1 H), 4.99 (d, $J = 4.0$, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 15.8, 16.8, 17.1, 18.6, 18.7, 30.1, 30.2, 32.3, 52.1, 74.9, 77.2, 77.3, 168.7, 169.5, 174.7; FABMS *m/z* 333 $[M + H]^+$ (52), 233 (100); HRFABMS *m/z* 333.1912 (calcd for $C_{16}H_{29}O_7$ $[M + H]^+$ 333.1913). Anal. Calcd for $C_{16}H_{28}O_7$: C, 57.82; H, 8.49. Found: C, 58.08; H, 8.63.

D-Mandelyl-L-mandelic Acid Methyl Ester (19a). 19a was obtained by coupling between L-**8a** (50 mg, 0.30 mmol) and D-**5a**, as described above for **14a**, followed by deprotection of the resulting Fmoc-protected didepside with piperidine/THF according to the general procedure to give a pale yellow solid (68 mg, 76%): mp = 85−86 °C; $[\alpha]_D = +57.8$ ($c = 9.5$, $CHCl_3$); IR (NaCl) ν_{max} 3420 br, 3065, 3033, 2953, 1746, 1453, 1437, 1217, 1172, 1093, 1067, 1028, 734, 696 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 3.40 (s or d, $J = 5.5$, 1 H), 3.73 (s, 3 H), 5.40 (s or d, $J = 5.5$, 1 H), 5.96 (s, 1 H), 7.27−7.61 (m, 10 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 52.8, 73.0, 75.4, 126.7, 127.2, 127.6,

127.9, 128.6, 128.8, 129.3, 133.1, 137.7, 168.7, 172.9; FABMS *m/z* 323 $[M + Na]^+$ (55), 301 $[M + H]^+$ (75), 283 (100), 261 (86); HRFABMS *m/z* 301.1077 (calcd for $C_{17}H_{17}O_5$ $[M + H]^+$ 301.1076).

***O*$^{\alpha}$-Methyl-L-mandelyl-D-mandelyl-L-mandelic Acid Methyl Ester (20a). (a) Preparation of 20a as a Reference Compound by Coupling between 19a and *O*$^{\alpha}$-Methyl-L-mandelic Acid (MPA).** A solution of **19a** (68 mg, 0.23 mmol), *O*$^{\alpha}$-methyl-L-mandelic acid (45 mg, 0.27 mmol), DIC (50 μL, 0.32 mmol), and DMAP (3 mg, 0.02 mmol) in THF (0.8 mL) was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and purified by flash chromatography with hexane/EtOAc (7:3) to give **20a** (89 mg, 88%) as an amorphous solid. **(b) By Methylation of 17a.** A mixture of **17a** (29 g, 0.07 mmol) and Ag_2O (8 mg, 0.04 mmol) in MeI (1 mL) was refluxed (50 °C) for 4 h. The mixture was evaporated to dryness, redissolved in $CHCl_3$, and evaporated twice to ensure complete removal of all the MeI (caution: MeI is volatile and carcinogenic! Work in a well-ventilated hood!). The residue was purified by flash chromatograpy from hexane/EtOAc (7:3) and HPLC (μ-Porasil, hexane/EtOAc (8:2), 2.0 mL/min) to give **20a** (23 mg, 76%) as an amorphous solid: $[\alpha]_D = +33.2$ ($c = 6.0$, $CHCl_3$); IR (NaCl) ν_{max} 3062, 3033, 2949, 2833, 1755, 1495, 1451, 1352, 1205, 1166, 1108, 1026, 737, 697 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.33 (s, 3 H), 3.69 (s, 3 H), 4.90 (s, 1 H), 5.96 (s, 1 H), 6.13 (s, 1 H), 7.29−7.43 (m, 15 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 52.7, 57.5, 74.7, 75.2, 82.3, 127.4, 127.6, 127.9, 128.7, 128.7, 128.9, 129.2, 129.3, 133.0, 133.3, 135.7, 167.5, 168.3, 170.0; FABMS *m/z* 471 $[M + Na]^+$ (100), 449 $[M + H]^+$ (36), 447 (33), 417 (43), 317 (25), 283 (50); HRFABMS *m/z* 449.1599 (calcd for $C_{26}H_{25}O_7$ $[M + H]^+$ 449.1600).

***O*$^{\alpha}$-Methyl-L-mandelyl-L-mandelyl-L-mandelic Acid Methyl Ester (21a). (a) Preparation of 20a as a Reference Compound by Coupling between 15a and *O*$^{\alpha}$-Methyl-L-mandelic Acid.** A solution of **15a** (45 mg, 0.15 mmol), *O*$^{\alpha}$-methyl-L-mandelic acid (30 mg, 0.18 mmol), DIC (33 μL, 0.21 mmol), and DMAP (2 mg, 0.01 mmol) in THF (0.8 mL) was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and purified by flash chromatography from hexane/EtOAc (7:3) to give **21a** (56 mg, 84%) as an amorphous solid. **(B) By Methylation (See above, Methylation of 20a) of 18a (54 mg, 0.12 mmol) To Give an Amorphous Solid (44 mg, 80%):** $[\alpha]_D = +111.5$ ($c = 6.0$, $CHCl_3$); IR (NaCl) ν_{max} 3061, 3030, 2923, 2851, 1754, 1494, 1450, 1348, 1205, 1163, 1107, 1038, 736, 696 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.50 (s, 3 H), 3.55 (s, 3 H), 4.93 (s, 1 H), 5.92 (s, 1 H), 6.13 (s, 1 H), 7.29−7.51 (m, 15 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 52.4, 57.6, 74.5, 75.2, 82.4, 127.4, 127.5, 127.9, 128.6, 128.7, 128.8, 128.8, 129.3, 129.4, 133.0, 133.1, 135.7, 167.5, 168.3, 170.1; FABMS *m/z* 471 $[M + Na]^+$ (100), 449 $[M + H]^+$ (16), 447 (19), 417 (22), 317 (18), 283 (62); HRFABMS *m/z* 449.1599 (calcd for $C_{26}H_{25}O_7$ $[M + H]^+$ 449.1600). Anal. Calcd for $C_{26}H_{24}O_7$: C, 69.62; H, 5.40. Found: C, 69.81; H, 5.52.

***O*$^{\alpha}$-Methyl-D-mandelyl-L-mandelyl-L-mandelic Acid Methyl Ester (22a). Preparation of 21a as a Reference Compound by Coupling between 19a and *O*$^{\alpha}$-Methyl-D-mandelic Acid. 22a** was prepared as described above for **21a**, with *O*$^{\alpha}$-methyl-D-mandelic acid, as an amorphous solid (59 mg, 87%): $[\alpha]_D = +62.8$ ($c = 5.7$, $CHCl_3$); IR (NaCl) ν_{max} 3063, 3032, 2949, 2833, 1757, 1495, 1449, 1209, 11647, 1108, 1040, 744, 699 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.49 (s, 3 H), 3.58 (s, 3 H), 4.99 (s, 1 H), 5.98 (s, 1 H), 6.11 (s, 1 H), 7.30−7.47 (m, 15 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 52.4, 57.6, 74.5, 75.3, 82.2, 127.3, 127.4, 127.5, 127.6, 127.9, 128.6, 128.8, 128.9, 129.3, 129.4, 132.8, 133.1, 135.7, 167.8, 168.3, 170.1; FABMS *m/z* 471 $[M + Na]^+$ (100), 449 $[M + H]^+$ (85), 417 (42); HRFABMS *m/z* 449.1599 (calcd for $C_{26}H_{25}O_7$ $[M + H]^+$ 449.1600).

***O*$^{\alpha}$-Tetrahydropyranyl-L-mandelyl-L-mandelic Acid (23a).** A solution of L-**8a** (100 mg, 0.60 mmol), L-**7a** (170 mg, 0.72 mmol), DIC (131 μL, 0.84 mmol), and DMAP (7 mg, 0.06 mmol) in THF (2 mL) was stirred at room temperature for 2 h. The reaction mixture was filtered and concentrated under reduced pressure. Purification by flash chromatography with hexane/EtOAc (7:3) gave **23a** (214 mg, 93%) as a sticky oil: IR (NaCl) ν_{max} 2948, 2856, 1755, 1448, 1267, 1210, 1161, 1125, 1074, 1032, 975, 733, 700 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.45−1.95 (m, 6 H), 3.45−3.54 (m, 1 H), 3.57 and 3.61 (s, 3 H), 3.65−3.78 and 3.91−3.99 (m, 1 H), 4.65 and 5.02 (t, J = 2.9, 1 H), 5.36 and 5.48 (s, 1 H), 5.96 and 5.97 (s, 1 H), 7.36−7.58 (m, 10 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 18.7, 25.3, 30.1, 52.4, 61.9, 74.8, 75.6, 76.4, 96.4, 97.2, 127.3, 127.4, 127.5, 127.7, 128.4, 128.5, 128.6, 128.7, 129.2, 133.4, 133.6, 135.8, 136.1, 168.6, 169.9, 170.7; FABMS *m/z* 407 $[M + Na]^+$ (26), 385 $[M + H]^+$ (65), 301 (59); HRFABMS *m/z* 385.1650 (calcd for $C_{22}H_{25}O_6$ $[M + H]^+$ 385.1651). Anal. Calcd for $C_{22}H_{24}O_6$: C, 68.72; H, 6.30. Found: C, 68.63; H, 6.37.

***O*$^{\alpha}$-Tetrahydropyranyl-L-mandelyl-L-mandelyl-L-mandelic Acid (24a).** A solution of L-**15a** (152 mg, 0.51 mmol), L-**7a** (143 mg, 0.61 mmol), DIC (111 μL, 0.71 mmol), and DMAP (6 mg, 0.05 mmol) in THF (1.5 mL) was stirred at room temperature for 2 h. The reaction mixture was filtered and concentrated under reduced pressure. Purification by flash chromatography from hexane/EtOAc (7:3) gave **24a** (233 mg, 89%) as a sticky oil: IR (NaCl) ν_{max} 3067, 3031, 2947, 2869, 1756, 1496, 1450, 1347, 1266, 1205, 1164, 1034, 973, 908, 738, 698 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.50−1.95 (m, 6 H), 3.43−3.55 (m, 1 H), 3.53 and 3.55 (s, 3 H), 3.69−3.77 and 3.82−3.95 (m, 1 H), 4.64 (t, J = 3.2), 5.03 (t, J = 2.8, 1 H), 5.37 and 5.49 (s, 1 H), 5.91 and 5.93 (s, 1 H), 6.09 and 6.12 (s, 1 H), 7.28−7.58 (m, 15 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 18.6, 18.8, 25.3, 29.7, 30.1, 52.4, 61.9, 62.0, 74.5, 74.6, 75.08, 75.13, 75.6, 96.5, 97.2, 127.4, 127.5, 127.7, 127.8, 128.4, 128.5, 128.6, 128.7, 129.3, 133.0, 133.1, 135.7, 136.1, 167.4, 168.2, 169.9, 170.7; FABMS *m/z* 519 $[M + H]^+$ (16), 435 (100); HRFABMS *m/z* 519.2018 (calcd for $C_{30}H_{31}O_8$ $[M + H]^+$ 519.2019). Anal. Calcd for $C_{30}H_{30}O_8$: C, 69.47; H, 5.83. Found: C, 69.68; H, 5.76.

H-[L-Man-L-Man]$_4$-OH (25). Wang resin (1.0 g, 1.0 mmol/g loading level) was placed into a 50 mL reaction vessel of a peptide synthesizer and subsequently submitted to eight cycles of the following: (1) depside coupling according to general procedure A with *O*$^{\alpha}$-THP-L-mandelic acid (710 mg, 3 mmol), DIC (470 μL, 3 mmol), and DMAP (12 mg, 0.1 mmol); (2) THP deprotection according to general procedure C. After each depside coupling step a small sample of resin was taken and submitted to the test described previously and the coupling repeated if necessary. The product was cleaved from the solid support by treatment with 10 mL of TFA/CH_2Cl_2 (1:1) for 1 h. The resin was filtered off and washed five times with CH_2Cl_2 (10 mL). The combined rinses were washed twice with 50 mL of water and dried over Na_2SO_4, and the solvent was removed under reduced pressure to give 1152 mg of crude **25**. RP-HPLC (Nucleosil, MeCN/water (70:30) with 0.1% TFA, 2.0 mL/min) of 115 mg of crude **25** afforded 81 mg (0.074 mmol, 74%) of octadepside **25** (t_R = 17 min) as a white solid: IR (NaCl) ν_{max} 3450 br, 3035, 2948, 2869, 1748, 1496, 1453, 1254, 1208, 1173, 1062, 1032, 737, 697 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.28 (s, 1 H), 5.83 (s, 1 H), 5.97 (s, 1 H), 6.00 (s, 1 H), 6.01 (s, 1 H), 6.02 (s, 1 H), 6.06 (s, 1 H), 6.13 (s, 1 H), 7.18−7.43 (m, 40 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 73.1, 74.6, 74.8, 75.3, 126.9, 127.4, 127.7, 127.8, 127.9, 128.6, 128.7, 128.8, 129.4, 132.3, 132.5, 137.4, 166.9, 172.6; FABMS *m/z* 1113 $[M + Na]^+$ (100), 1091 $[M + H]^+$ (27). Anal. Calcd for $C_{64}H_{50}O_{17}$: C, 70.44; H, 4.62. Found: C, 70.10; H, 4.78.

H-[D-Man-L-Man]$_4$-OH (26). Octadepside **26** was synthesized as described above for octadepside **25**, starting with 1.0 g of Wang resin and carrying out eight coupling−deprotection cycles using, alternately, *O*$^{\alpha}$-THP-L- and *O*$^{\alpha}$-THP-D-mandelic acid (710 mg, 3.0 mmol) in the coupling reactions. The crude product, a white foam, was purified by RP-HPLC (Partisil, MeCN/water (70:30) with 0.1% TFA, 2.0 mL/min) to obtain 817 mg (0.75 mmol, 75%) of octadepside **2** (t_R = 16 min 40 s) as a white solid: $[\alpha]_D = +17.3$ (acetone, c = 2.4); IR (NaCl) ν_{max} 3500 br, 3061, 3032, 2948, 1750, 1494, 1452, 1247, 1201, 1167, 1046, 1027, 734, 696 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 5.36 (s, 1 H), 5.99 (s, 1 H), 6.17 (s, 1 H), 6.19 (s, 3 H), 6.21 (s, 1 H), 6.23 (s, 1 H), 7.22−7.46 (m, 40 H); ^{13}C NMR (63 MHz, acetone-d_6) δ 73.1, 74.4, 74.8, 74.9, 75.1, 127.0, 127.4,

127.7, 128.1, 128.3, 128.6, 129.0, 129.2, 133.2, 133.3, 133.5, 134.0, 139.0, 166.7, 166.8, 167.0, 167.2, 168.4, 171.7; FABMS *m/z* 1113 $[M + Na]^+$ (100), 979 (10), 663 (8), 491 (12), 403 (22), 357 (34); HRFABMS *m/z* 1113.3000 (calcd for $C_{64}H_{50}O_{17}Na_1$ $[M + Na]^+$ 1113.2946). Anal. Calcd for $C_{64}H_{50}O_{17}$: C, 70.44; H, 4.62. Found: C, 70.15; H, 4.80.

H-[L-Lac-L-Hiv]$_3$-OH (27). Hexadepside **27** was synthesized as described above for octadepside **25**, starting with 1.0 g of Wang resin and carrying out six coupling−deprotection cycles using, alternately, O^{α}-THP-L-α-hydroxyisovaleric acid (606 mg, 3.0 mmol) and O^{α}-THP-L-lactic acid (522 mg, 3.0 mmol) in the coupling reactions. The crude product, a white foam, was purified by RP-HPLC (Nucleosil, MeCN/water (50:50) with 0.1% TFA, 1.8 mL/min) to obtain 384 mg (0.72 mmol, 72%) of hexadepside **3** (t_R = 24 h 45 min) as a white solid: $[\alpha]_D = -83.9$ (acetone, $c = 1.8$); IR (NaCl) ν_{max} 3480 br, 2970, 2942, 1739, 1468, 1208, 1134, 1098, 1033 cm^{-1}; ^1H NMR (300 MHz,$CDCl_3$) δ 0.99−1.06 (m, 18 H), 1.50 (d, J = 6.8, 3 H), 1.57 (d, J = 6.8, 3 H), 1.60 (d, J = 6.8, 3 H), 2.22−2.46 (m, 3 H), 4.39 (q, J = 6.9, 1 H), 4.36 (d, J = 3.9, 1 H), 4.98 (d, J = 3.9, 1 H), 4.99 (d, J = 3.9, 1 H), 5.18 (q, J = 6.9, 1 H), 5.21 (q, J = 6.9, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 16.6, 16.7, 16.8, 16.9, 18.5, 18.6, 18.7, 20.4, 29.9, 30.0, 66.7, 68.9, 69.0, 76.8, 76.9, 77.1, 168.4, 169.9, 173.7, 175.4; FABMS *m/z* 557 $[M + Na]^+$ (100), 535 $[M + H]^+$ (7), 337 (19), 245 (39); HRFABMS *m/z* 557.2189 (calcd for $C_{24}H_{38}O_{13}Na_1$ $[M^+ + Na]$ 557.2210). Anal. Calcd for $C_{24}H_{38}O_{13}$: C, 53.91; H, 7.17. Found: C, 54.01; H, 7.23.

H-[L-Man]$_6$-OH (28). Hexadepside **28** was synthesized as described above for octadepside **25**, starting with 1.0 g of Wang resin and carrying out six coupling−deprotection cycles using O^{α}-THP-L-mandelic acid (710 mg, 3.0 mmol) in the coupling reactions. The crude product, a white foam, was purified by RP-HPLC (Partisil, MeCN/water (65:35) with 0.1% TFA, 2.0 mL/min) to obtain 690 mg (0.84 mmol, 84%) of hexadepside **5** (t_R = 15.5 min) as a white solid: IR (NaCl) ν_{max} 3034, 2948, 2869, 1752, 1497, 1454, 1254, 1206, 1167, 1032, 737, 696 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 5.36 (s, 1 H), 5.88 (s, 1 H), 6.11 (s, 1 H), 6.14 (s, 1 H), 6.15 (s, 1 H), 6.16 (s, 1 H), 7.31−7.55 (m, 30 H); ^{13}C NMR (63 MHz, acetone-d_6) δ 72.9, 74.4, 74.7, 75.1, 127.0, 127.6, 128.0, 128.1, 128.2, 128.5, 128.6, 129.1, 129.2, 129.3, 133.0, 133.1, 133.4, 133.9, 138.9, 166.8, 166.9, 167.3, 168.3, 171.9; FABMS *m/z* 867 $[M + 2Na]^+$ (14), 845 $[M + Na]^+$ (100), 800 (9), 711 (9), 583 (9), 557 (36), 329 (25); HRFABMS *m/z* 845.2208 (calcd for $C_{48}H_{38}O_{13}Na_1$ $[M + Na]^+$ 845.2210). Anal. Calcd for $C_{48}H_{38}O_{13}$: C, 70.07; H, 4.65. Found: C, 70.22; H, 4.81.

Cyclo[(L-Man)$_5$-D-Man] (29). To a suspension of hexadepside **28** (411 mg, 0.5 mmol), triphenylphosphine (170 mg, 0.65 mmol), and $NaClO_4$ (61 mg, 0.5 mmol) in toluene (15 mL) at 0 °C was added a solution of DEAD (101 μL, 0.65 mmol) in 0.5 mL of toluene by syringe. The mixture was maintained for 15 min at 0 °C and then allowed to warm to room temperature, and stirring was continued for a further 8 h. The mixture was filtered and concentrated under reduced pressure, and the crude product was purified by flash chromatography (hexane/EtOAc (65:35) with 0.3% *i*-PrOH) to give **4** (60 mg, 15%) as a white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 5.94 (s, 1 H), 6.04 (s, 1 H), 6.06 (s, 1 H), 6.10 (s, 1 H), 6.15 (s, 2 H), 7.17−7.48 (m, 30 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 74.5, 75.3, 75.5, 75.8, 76.4, 77.0, 127.5, 127.9, 128.0, 128.2, 128.4, 129.0, 129.2, 129.5, 129.7, 129.8, 129.9, 130.0, 131.4, 132.2, 132.5, 133.0, 133.3, 133.5, 166.7, 166.9, 167.1, 167.8, 167.9, 168.3; FABMS *m/z* 827 $[M + Na]^+$ (100), 355 (8), 327 (17), 281 (33); HRFABMS *m/z* 827.2074 (calcd for $C_{48}H_{36}O_{12}Na_1$ $[M + Na]^+$ 827.2104). Anal. Calcd for $C_{48}H_{36}O_{12}$: C, 71.64; H, 4.51. Found: C, 71.51; H, 4.66.

Cbz-[L-Val-D-Man-D-Val-L-Lac]$_3$-OH (31). Wang resin (0.5 g, 1.0 mmol/g loading level) was placed into a 25 mL reaction vessel of a peptide synthesizer and washed with 10 mL of THF. The first unit, O^{α}-THP-L-lactic acid (**7c**) (261 mg, 1.5 mmol), was coupled to the resin according to general procedure A with DIC (235 μL, 1.5 mmol) and DMAP (6 mg, 0.05 mmol), followed by THP deprotection according to general procedure C. The resin was subsequently submitted to the following series of four coupling−deprotection cycles: (i) depside coupling according to general procedure A with N^{α}-Bpoc-D-valine[31] (533 mg, 1.5 mmol), DIC (235 μL, 1.5 mmol), and DMAP (6 mg, 0.05 mmol), followed by Bpoc deprotection according to general procedure D; (ii) peptide coupling according to general procedure B with O^{α}-THP-D-mandelic acid (D-**7a**) (355 mg, 1.5 mmol) and DIC (235 μL, 1.5 mmol) followed by THP deprotection according to general procedure C; (iii) depside coupling according to general procedure A with N^{α}-Bpoc-L-valine[31] (533 mg, 1.5 mmol), DIC (235 μL, 1.5 mmol), and DMAP (6 mg, 0.05 mmol), followed by Bpoc deprotection according to general procedure D; and (iv) peptide coupling according to general procedure B with D-**7a** (261 mg, 1.5 mmol) and DIC (235 μL, 1.5 mmol) followed by THP deprotection according to general procedure C. The whole series, from i to iv, was effected three times, except for the last peptide coupling (iv), which was only carried out twice. In the last depside coupling (iii), N^{α}-Cbz-L-valine was used instead of the Bpoc derivative. Depside couplings were monitored by the test described previously and peptide couplings with the ninhydrin test,[38] and in both cases, couplings were repeated if necessary. The product was cleaved from the solid support by treatment with 10 mL of TFA/CH_2Cl_2 (1:1) for 1 h. The resin was filtered and washed five times with CH_2Cl_2 (10 mL). The combined rinses were washed twice with 50 mL of water and dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude depsipeptide was purified by RP-HPLC (Nucleosil, MeCN/water (62:38) with 0.1% TFA, 2.0 mL/min) to obtain 429 mg (0.31 mmol, 63%) of protected depsipeptide **6** (t_R = 43 h 55 min) as a white solid: $[\alpha]_D$ −36.0 ($CHCl_3$, $c = 4.0$); IR (NaCl) ν_{max} 3320 br, 3035, 2968, 2933, 2871, 1747, 1670, 1536, 1167, 1145, 755, 697, 667, 481 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3 + D_2O$) δ 0.84−1.17 (m, 36 H), 1.50 (td, 9 H), 2.06−2.35 (m, 6 H), 4.06−4.57 (m, 6 H), 5.04 (d, J = 12.2, 1 H), 5.23 (d, J = 12.2, 1 H), 5.17 (q, J = 7.1, 1 H), 5.38 (q, J = 6.5, 1 H), 6.00 (d, J = 7.8, 1 H), 6.14 (s, 1 H), 6.17 (s, 1 H), 6.21 (s, 1 H), 7.36−7.55 (m, 20 H), 7.76 (m, 3 H), 7.90 (d, J = 6.3, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 16.8, 17.5, 17.6, 18.3, 18.4, 19.0, 19.1, 19.2, 29.2 (· 2), 29.3, 29.7, 30.0, 30.2, 57.8, 59.1, 59.5, 59.7, 59.8, 60.3, 67.4, 69.3, 70.5, 70.8, 75.6, 76.0, 77.3, 127.5, 127.6, 128.2, 128.5, 128.6, 128.7, 129.0, 134.9, 135.2, 135.3, 136.2, 157.2, 169.1, 170.1, 170.2, 170.4, 170.5, 170.6, 170.8, 171.4, 171.5, 171.8, 171.9, 172.0; FABMS *m/z* 1387 $[M + Na]^+$ (100), 1365 $[M + H]^+$ (20), 1316 (30). Anal. Calcd for $C_{71}H_{92}N_6O_{21}$: C, 62.43; H, 6.79; N, 6.16. Found: C, 62.58; H, 6.70; N, 6.04.

H-[L-Val-D-Man-D-Val-L-Lac]$_3$-OH (32). Depsipeptide **32** was synthesized as described above for **31**, starting with 0.3 g of Wang resin (1.0 mmol/g loading level) and using N^{α}-Bpoc-L-valine for the last depside coupling step. The crude product was purified by RP-HPLC (Partisil, MeCN/water (55:45) with 0.1% TFA, 2.5 mL/min) to obtain 189 mg (0.14 mmol, 47%) of **32** (t_R = 37 h 40 min) as a white solid: $[\alpha]_D$ −31.1 ($CHCl_3$, c = 1.0); IR (NaCl) ν_{max} 2969, 2937, 2878, 1748, 1665, 1534, 1460, 1195, 1145, 756, 699 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3 + D_2O$) δ 0.74−1.02 (m, 36 H), 1.33−1.52 (m, 9 H), 2.00−2.38 (m, 6 H), 4.03−4.66 (m, 6 H), 5.03 (q, J = 7.3, 1 H), 5.23−5.31 (m, 1 H), 6.08 (s, 1 H), 6.14 (s, 1 H), 6.21 (s, 1 H), 7.30−7.53 (m, 20 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 16.6, 17.5, 17.6, 17.8, 17.9, 18.0, 18.4, 18.6, 18.7, 18.8, 19.0, 29.3, 29.5, 29.7, 30.6, 57.2, 58.1, 58.2, 58.4, 58.5, 58.7, 69.8, 70.9, 72.0, 75.7, 76.1, 77.2, 127.3, 127.5, 127.6, 128.7, 128.8, 129.1, 129.2, 131.9, 134.5, 135.2, 166.9, 168.8, 169.0, 169.7, 170.9, 171.1, 171.2 (· 2), 171.3, 171.4, 171.8, 173.1; FABMS *m/z* 1253 $[M + Na]^+$ (20), 1231 $[M + H]^+$ (100), 1159 (17). Anal. Calcd for $C_{63}H_{86}N_6O_{19}$: C, 61.45; H, 7.04; N, 6.82. Found: C, 61.63; H, 6.91; N, 6.71.

Cyclo[L-Val-D-Man-D-Val-L-Lac]$_3$ (30). (a) Cyclization via the Acid Chloride. 32 (88 mg, 0.065 mmol) was dissolved in $SOCl_2$ (0.5 mL) and kept at room temperature for 30 min. The solution was concentrated under reduced pressure and redissolved twice in toluene. The resulting acid chloride was dissolved in 5 mL of toluene and added by syringe during 2 h to a stirred solution of DIEA (20 μL) in 5 mL of toluene. After

(38) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595−598.

24 h, the solvent was removed under reduced pressure. The residue was dissolved in ether and filtered. The ether solution was washed with 1 N HCl, $NaHCO_3$, and water, dried (Na_2SO_4), and filtered. Purification of the crude product by RP-HPLC (Nucleosil, MeCN/water (80:20) with 0.1% of TFA, 2.0 mL/min) gave 11 mg of **30** (t_R = 17 h 25 min) as a solid. **(b) Cyclization with HATU. 32** (99 mg, 0.074 mmol), HATU (56 mg, 0.147 mmol), and *N*,*N*-diisopropylethylamine (32 μL, 0.187 mmol) were dissolved in 75 mL of THF at 4 °C. The solution was stirred at 4 °C for 1 h and then allowed to warm to room temperature. After 24 h, the solvent was removed under reduced pressure. Elaboration and purification as described above yielded 21 mg of **30**: mp = 201−203 °C ($CHCl_3$); $[\alpha]_D$ = −21.8 ($CHCl_3$, c = 0.8); IR (NaCl) ν_{max} 3054, 2928, 2843, 1686, 1648, 1508, 1447, 1353, 1281, 1177, 1161, 1111, 1090, 823, 760, 720 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$ + D_2O) δ 0.85 (d, J = 6.6, 1 H), 0.91 (d, J = 6.6, 1 H), 0.97 (d, J = 6.6, 1 H), 1.10 (d, J = 6.6, 1 H), 1.50 (d, J = 6.9, 1 H), 2.13−2.36 (m, 6 H), 4.00 (dd, J_1 = 9.7, J_2 = 6.8, 1 H), 4.11 (dd, J_1 = 9.6, J_2 = 6.8, 1 H), 5.33 (q, J = 6.8, 1 H), 6.12 (s, 1 H), 7.29−7.36 (m, 3 H), 7.40−7.46 (m, 2 H), 7.92 (d, J = 6.7, 1 H), 8.06 (d, J = 6.7, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 17.4, 19.2, 19.3, 19.4 (· 2), 28.5, 28.7, 59.6, 59.8, 70.7, 76.0, 127.4, 128.4, 128.6, 135.6, 170.2, 170.9 (· 2), 172.3; FABMS m/z 1251 $[M + K]^+$ (36), 1235 $[M + Na]^+$ (36), 1213 $[M + H]^+$ (34). Anal. Calcd for $C_{63}H_{84}N_6O_{18}$: C, 62.36; H, 6.98; N, 6.93. Found: C, 62.18; H, 6.88; N, 7.01.

Acknowledgment. This work was supported by grants from CICYT (PM98-0227) and XUGA (20910B96 and 20908B97).

JO981580+