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Determination of the absolute stereochemistry of Etzionin

Esther Vaz, Miryam Fernandez-Suarez and Luis Muñoz*

Departamento de Química Orgánica, Facultade de Ciencias, Universidade de Vigo, 36200 Vigo, Spain

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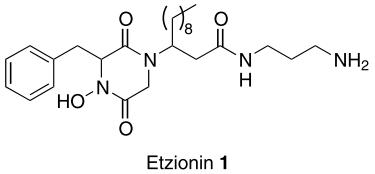
Abstract—The absolute configuration of Etzionin, a marine peptide-like compound isolated in 1989 from a red tunicate collected from the Red Sea has been determined by a combination of synthetic and spectroscopic procedures. Finally, its absolute stereochemistry has been established as 3*S*,3′*R*.

1. Introduction

Etzionin **1** is a nitrogen-containing metabolite that was isolated by Kashman et al. from an unidentified red tunicate collected in the Gulf of Eilat (the Red Sea).[1] Subsequent studies involving spectroscopic and spectrometric techniques in conjunction with chemical degradations established its structure as a highly functionalised 1,4-diketopiperazine formed by the formal condensation of *N*-hydroxyphenylalanine and *N*-(3-dodecanamido)glycine. However, the stereochemistry of the stereogenic centres of Etzionin **1** was not elucidated. Etzionin **1** was considered to be responsible for the antifungal activity of the tunicate from which it was isolated. On the basis of the novel structure of Etzionin—at least in terms of marine organisms—Kashman raised doubts about the origin of the metabolite since diketopiperazine hydroxamates are known fungal metabolites[2,3] (Fig. 1).

A few compounds that incorporate β-amino acids have been isolated and characterised during structural and synthetic studies of the metabolites of the tunicate *Didemnum rodriguesi* collected in New Caledonia (Pacific Sea).[4–6] During these ongoing structural studies, one of the metabolites was found to have the same structure as Etzionin **1**. The identification of the same natural product in two closely related species, which are separated by several thousand kilometres and exist in completely different environments, has re-opened the debate regarding the origin of Etzionin **1**.



Etzionin **1**

Figure 1.

This finding, together with the significant biological activity of **1**, prompted us to determine the absolute stereochemistry of the metabolite.

2. Results and discussion

We decided to carry out the stereochemical analysis by comparing the natural compound with the four possible stereoisomers synthesised as standards from enantiomerically pure starting materials.

It has been reported that the hydroxamate group is readily eliminated and this results in the loss of the stereochemical identity at C_3. For this reason we considered the preparation of compound **2**, a simpler, deoxygenated derivative of Etzionin. The synthesis of **2** was carried out both from the isolated natural product and by complete synthesis, using (3*S*)- and (3*R*)-3-methyl amino dodecanoate **4** and *L*- and *D*-phenylalanine **3** as starting materials (Fig. 2).

The derivative *nat*-**2** was prepared by reduction of the N–O bond in Etzionin **1** under mild conditions that involved $TiCl_3$ and controlled pH.[7] This methodology has been widely applied in the synthesis of β-lactams. Finally, the terminal amino group in the reduced product was acetylated to give the amide *nat*-**2** (Scheme 1).

* Corresponding author. E-mail: imunoz@uvigo.es

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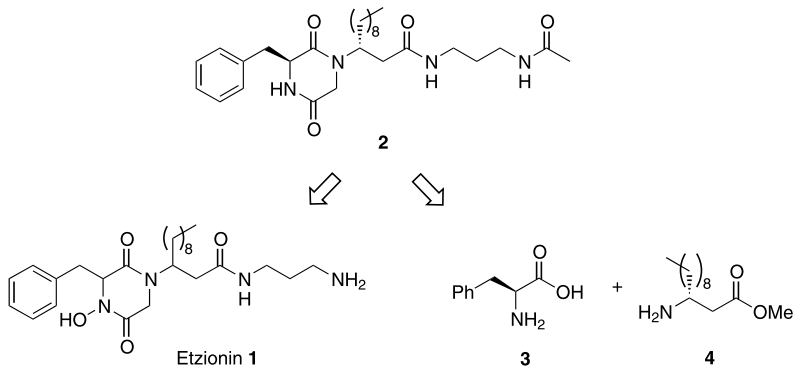
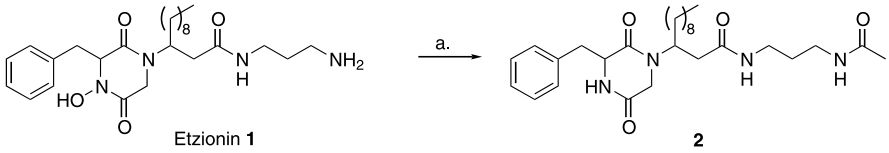
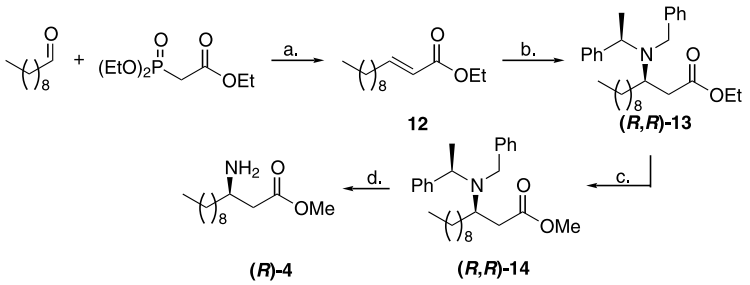


Figure 2.



Scheme 1. *Reagents and conditions*: (a) i. $TiCl_3$, ii. Ac_2O, pyridine (42%).

Once the naturally deoxygenated derivative *nat*-**2** had been obtained, it was possible to undertake the total synthesis of the four stereoisomers of **2**. The proposed starting materials were *L*- and *D*-phenylalanine **3**, both enantiomers of 3-aminododecanoic acid **4** and bromoacetic acid. A commercial source of the β-amino acid was not available and so it was necessary to carry out the enantioselective synthesis of this compound. Several approaches were followed in an attempt to achieve this goal. Initially, the two enantiomers were prepared following a route analogous to that used to prepare the β-amino acid of Minalemine A.[5] Although this route provided a reasonable amount of enantiomerically pure material, it was considered impractical for the synthesis of compounds with a single stereocentre. A number of routes to similar compounds have been published and so we decided to assess the practicality of several of these in terms of our synthetic aim. Firstly, we tried to prepare our target compounds from the chiral pool using serine and aspartic acids as starting materials.[8,9] Unfortunately, these routes did not provide any advantage over our existing method. In contrast, the well-documented method of Davies, which involves the use of benzyl phenyl ethyl amine and an unsaturated ester, proved to be very efficient.[10–13] Thus, a Horner–Wadsworth–Emmons reaction between decylaldehyde and the anion obtained by treatment of the corresponding phosphonoacetate with *n*-butyllithium provided a mixture of the unsaturated ethyl esters 2*E*-**12** and 2*Z*-**12** in a 12:1 ratio in good yield. Subsequent 1,4-addition of the (*R*)- and (*S*)-*N*-benzyl-*N*-phenylethyl lithium amides to **12** afforded the two enantiomers (*R*,*R*)-**13** and (*S*,*S*)-**13** in excellent yield and enantiomeric excess since a second set of signals corresponding to the diastereomers could not be detected by ^{1}H NMR. Transesterification of **13** was attempted under mild basic conditions using sodium acetate in methanol and also in anhydrous acidic conditions involving thionyl chloride in methanol. However, both of these approaches proved unsuccessful. Finally, the methyl esters **14** were obtained in high yield by treatment of **13** with sodium in methanol. Although the reaction conditions were harder than the previous ones, no epimerization was detected by ^{1}H NMR. Hydrogenolysis (60 psi) at 60°C led to cleavage of the *N*-benzyl and *N*-phenylethyl moieties to give the corresponding *R*- or *S*- β-amino esters **4** (Scheme 2). Alternatively, the last two steps could be carried out in



Scheme 2. *Reagents and conditions*: (a) *n*BuLi, THF, −78°C to rt (83%); (b) (1*R*)-*N*-benzyl-*N*-phenethylamine, *n*BuLi, THF, −78°C (99%); (c) Na, MeOH, 0°C to rt (99%); (d) H_2, Pd/C 10%, AcOH, 60°C (98%).

reverse order. Nevertheless, this approach did not provide any advantage since transesterification did not proceed under mild conditions and compounds **4** could only be obtained in good yields by treatment with sodium hydride in methanol.

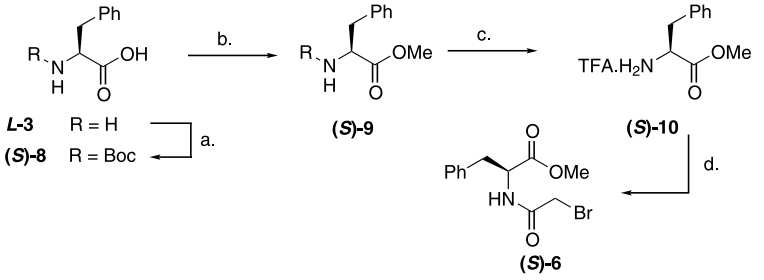
Once the building blocks had been obtained, we proceeded to assemble to desired structure. Phenylalanine was fully protected by firstly reacting it with Boc_2O in THF/water and then with iodomethane and sodium bicarbonate in DMF to afford **9**. After selective deprotection of the amino group, compound **10** was coupled with bromoacetic acid using DCC as a condensing agent to give the alkyl bromide **6** (Scheme 3).

Bromide **6** was then submitted to nucleophilic displacement with the β-amino acid methyl ester **4**, to give the peptide **5**. Remarkably, an excess of the chiral amine was not necessary because of the steric hindrance caused by the long aliphatic chain, a situation that precludes polyalkylation. Subsequent cyclization of **5** was the key step of the synthesis. This reaction has been carried out under reflux in different solvents (methanol and toluene) and at different concentrations. The best results were obtained when the cyclizations were carried out in a Schlenk tube using toluene and high dilution to give the 2,5-diketopiperazines in good yields.[14] The final step involved treatment of ester **11** with neat 1,3-propanediamine at 25°C. Acetylation of the free amino group with acetic anhydride in pyridine provided the target compounds **2**. All the compounds **5**, **11** and **2** were found diastereomerically pures since no diastereomers could be found by ^1H NMR in their respective reaction crudes (Scheme 4).

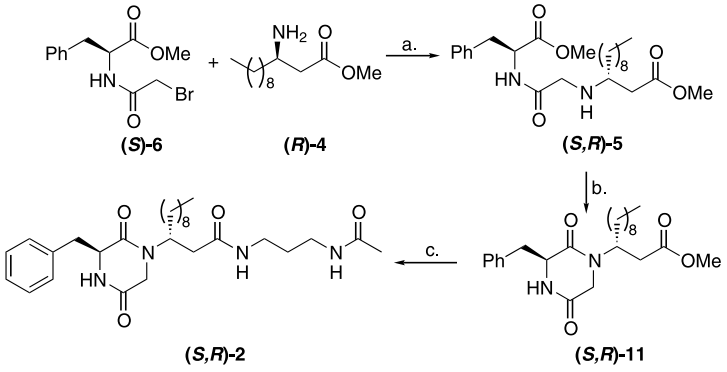
In order to obtain the four possible stereoisomers of **2** as standards, the synthesis of both enantiomers of every building block was performed and these were assembled in the corresponding four different combinations to give (*R*,*R*)-, (*R*,*S*)-, (*S*,*R*)- and (*R*,*R*)-**2**. Although one would expect the reactivity of these systems to be very similar, consistently higher conversion yields were obtained for the *S*,*S* diastereomer in comparison with the *R*,*S* analogue in the cyclization step.

Comparison of the ^1H NMR spectra of *nat*-**2** and diastereomers (*R**,*S**)-**2** and (*R**,*R**)-**2** showed very similar spectra for *nat*-**2** and (*R**,*S**)-**2**, whereas the two diastereomeric standards have significantly different spectra. In order to remove any ambiguity due to the small differences in chemical shifts of *nat*-**2** and (*R**,*S**)-**2**, a ^1H NMR spectrum of a mixture of *nat*-**2** and (3*S*,3′*R*)-**2** was performed. This spectrum showed only a single set of signals with no additional splitting. Comparison of the spectroscopic data was also made through *J*-correlated 2D NMR experiments (COSY, HMQC, HMBC), and this technique confirmed the relative configuration (*R**,*S**) for the natural product.

Comparison of *nat*-**2** with the synthetic stereoisomeric standards in a chiral environment was necessary to establish the absolute stereochemistry of the natural product. Initially, CD was considered the method of choice since it has proved to be a very valuable tool to

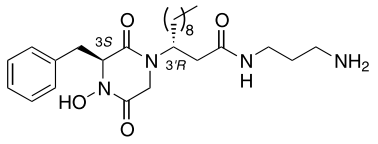


Scheme 3. *Reagents and conditions*: (a) Boc_2O, Et_3N, THF/H_2O (99%); (b) MeI, $NaHCO_3$, DMF (97%); (c) TFA, CH_2Cl_2, 0°C (93%); (d) $BrCH_2COOH$, DIEA, DCC, DMAP, CH_2Cl_2 (64%).



Scheme 4. *Reagents and conditions*: (a) DIEA, DMF, 0°C to rt (89%); (b) toluene, 120°C (40%); (c) i. 1,3-propanediamine, 25°C, ii. Ac_2O, Et_3N or AcCl, Et_3N, CH_2Cl_2, 25°C.

establish the absolute stereochemistry of the Minalemines. Surprisingly, both the synthetic standards **2** and the derivative *nat*-**2** were found to be inactive with this technique. In addition, attempts to separate the enantiomeric standards by chiral HPLC proved impossible with a variety of columns. As an alternative, the optical rotations of *nat*-**2**, (*R*,*S*)-**2** and (*S*,*R*)-**2** were measured. The specific rotation values were −51.9 (*c* 0.08, $CHCl_3$), +53.5 (*c* 0.11, $CHCl_3$) and −53.1 (*c* 0.06, $CHCl_3$), respectively. At first glance, the value obtained for *nat*-**2** is slightly lower than those obtained for the standards. However, all three values are in good agreement within the limits of the possible errors in the measurement. This result provides further evidence that the absolute configuration of Etzionin **1** is 3*S*,3′*R*. At this point it is worth pointing out that the absolute configuration *R* found for the β-amino acid of Etzionin **1** is the same as that previously established for the β-amino acids of Minalemines, which are similar peptide-like compounds found in the extracts of the same organism, *Didemnum rodriguesi* (Fig. 3).



Etzionin **1**

Figure 3.

3. Experimental

3.1. General

THF was distilled over Na/benzophenone immediately prior to use. CH_2Cl_2 and toluene were distilled over P_2O_5. MeOH was distilled over Mg/I_2. Flash chromatography was carried out with silica gel 60 (230–400 mesh). TLC was performed with silica gel 60 F_{254} on coated aluminium plates. Compounds were visualized using UV light, iodine vapour, aqueous $KMnO_4$ solution, or an ethanolic ninhydrin solution. Semipreparative HPLC purifications were performed using a system with a μPorasyl column (7.8×300 mm) for normal phase separations and a differential refractometer as the detector. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ and CD_3OD solutions on Bruker AMX-400 and ARX-400 spectrometers. All chemical shifts (δ) are reported in ppm relative to TMS using the residual solvent signal as internal reference: δ 7.26 and 77.0 ppm for the $CHCl_3$ ^1H and ^{13}C signals, respectively, and δ 3.30 and 49.05 ppm for the MeOH ^1H and ^{13}C signals, respectively. Coupling constants (J) are given in Hz. Optical rotation data were recorded on an Autopol-IV Rudolph digital polarimeter using a cell of 1 dm path length. Melting points were recorded on a Gallenkamp apparatus and are uncorrected. IR spectra were recorded using an FT-IR apparatus. Mass spectra were recorded on an HP5989A spectrometer (70 eV).

3.2. (2*S*)-*N*-Bromoacetylphenylalanine methyl ester, (*S*)-6

To a solution of trifluoroacetate (*S*)-**10** (508.0 mg, 1.73 mmol) in CH_2Cl_2 (2.5 mL) at 0°C was added diisopropylethylamine (0.3 mL, 1.73 mmol). The mixture was stirred for 25 min and then added to a solution of 2-bromoacetic acid (333.5 mg, 2.40 mmol) in CH_2Cl_2 (2.5 mL). DMAP (10.5 mg) and DCC (392.0 mg, 1.90 mmol) were added and the reaction mixture was stirred for 17.5 h at 25°C. The reaction mixture was filtered and concentrated. The residue was purified by chromatography (silica gel, hexane/EtOAc 15% to 25%) to afford 332.0 mg (64%) of a white solid. mp 86.5–87.5°C; $[\alpha]_D^{23}=+50.6$ (*c* 1.05, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.34–7.25 (m, 3H, Ph), 7.13 (d, $J=6.8$ Hz, 2H, Ph), 6.84 (bs, 1H, NH), 4.86 (q, $J=5.8$ Hz, 1H, H_2), 3.87 (d, $J=13.6$ Hz, 1H, $H_{2'}$), 3.84 (d, $J=13.7$ Hz, 1H, $H_{2'}$), 3.75 (s, 3H, CH_3O–), 3.21–3.11 (m, 2H, H_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.2 (s), 165.1 (s), 135.3 (s), 129.2 (d), 128.6 (d), 127.3 (d), 53.6 (d), 52.4 (q), 37.6 (t), 28.6 (t); MS (FAB^+): m/z (%) 303 (M^++3, 10), 302 (M^++2, 68), 301 (M^++1, 12), 300 (M^+, 71), 242 (18), 240 (18); HRMS (FAB^+): calcd for $C_{12}H_{15}NO_3Br$ 300.0235; found 300.0238; IR (NaCl, cm^{-1}): 3302, 3065–2855, 1744, 1663.

3.3. (2*R*)-*N*-Bromoacetylphenylalanine methyl ester, (*R*)-6

The spectroscopic data were identical to those for the corresponding (*S*)-**6** enantiomer. $[\alpha]_D^{23}=-56.6$ (*c* 0.76, $CHCl_3$).

3.4. (2*E*)-Dodec-2-enoic acid ethyl ester, 12

To a solution of triethylphosphonoacetate (8.00 g, 35.68 mmol) in THF (45.0 mL) at −78°C was added 1.49 M *n*BuLi in hexane (23.9 mL, 35.68 mmol). The reaction mixture was stirred for 30 min and a solution of decyl aldehyde (5.2 mL, 27.45 mmol) in THF (46.0 mL) was added dropwise at −78°C. The resulting mixture was stirred at −78°C for 1.5 h and then allowed to warm up to rt. 10% HCl was added until neutral pH was attained and the product was extracted with *t*BuOMe (3×). The combined organic layers were washed with H_2O (3×) and aqueous $NaHCO_3$ (1×), dried (Na_2SO_4) and evaporated. The residue was purified by chromatography (silica gel, hexane/EtOAc 2%) to give 5.19 g (83%) of a yellow oil, *E*/*Z* 12.5:1. ^1H NMR ($CDCl_3$, 400 MHz): δ 6.95 (dt, $J=15.6$ and 7.0 Hz, 1H, H_3), 5.79 (dt, $J=15.6$ and 1.5 Hz, 1H, H_2), 4.16 (q, $J=12.3$ Hz, 2H, $CH_3C\underline{H}_2O$–), 2.18 (m, 2H, H_4), 1.39 (m, 2H, H_5), 1.29–1.24 (m, 15H, H_{6-11} and $C\underline{H}_3CH_2O$–), 0.86 (t, $J=6.8$ Hz, 3H, H_{12}); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 166.6 (s), 149.3 (d), 121.2 (d), 59.9 (t), 32.1 (t), 31.8 (t), 29.4 (t), 29.3 (t), 29.2 (t), 29.1 (t), 28.0 (t), 22.6 (t), 14.2 (q), 14.0 (q); MS (EI^+): m/z (%) 227 (M^++1, 9), 226 (M^+, 8), 199 (17), 182 (13), 181 (100), 180 (32), 155 (13), 138 (34), 127 (33), 101 (95); HRMS (EI^+): calcd for $C_{14}H_{26}O_2$ 226.1933; found 226.1928; IR (NaCl, cm^{-1}): 2927, 2855, 1724, 1655.

3.5. Ethyl (3*R*)-3-{benzyl[(1*R*)-1-phenylethyl]amino}-dodecanoate, (*R*,*R*)-13

To a solution of benzyl[(1*R*)-1-phenylethyl]amine (1.49 g, 7.07 mmol) in THF (25.0 mL) was added *n*BuLi (1.44 M in hexane, 4.6 mL) at −78°C and the reaction mixture was stirred for 30 min. A solution of 2*E*-dodec-2-enoic acid ethyl ester **12** (1.00 g, 4.42 mmol) in THF (7.4 mL) was added dropwise at −78°C and the resulting mixture was stirred for 2.5 h. A solution of 2,6-di-*tert*-butylphenol (273.4 mg, 13.25 mmol) in THF (4.4 mL) was added, the mixture was allowed to reach rt and the solvent was evaporated. The crude material was washed with brine (1×) and extracted with CH_2Cl_2 (3×). The combined organic layers were dried (Na_2SO_4) and evaporated. The residue was purified by chromatography (silica gel, hexane to hexane/EtOAc 25%) to afford 1.91 g (99%) of a yellow oil. $[\alpha]_D^{20}=+7.7$ (*c* 0.04, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.41–7.19 (m, 10H, 2Ph), 4.03 (m, 2H, $CH_3C\underline{H}_2O$–), 3.84 (m, 1H, $PhCH_3C\underline{H}$–), 3.78 (d, $J=14.9$ Hz, 1H, $PhC\underline{H}_2$–), 3.53 (d, $J=14.9$ Hz, 1H, $PhC\underline{H}_2$–), 3.29 (m, 1H, H_3), 2.06 (dd, $J=14.4$ and 4.5 Hz, 1H, H_2), 1.98 (dd, $J=14.4$ and 8.5 Hz, 1H, H_2), 1.50 (m, 2H, H_4), 1.32 (d, $J=6.9$ Hz, 3H, $PhC\underline{H}_3CH$–), 1.25 (bs, 14H, H_{5-11}), 1.17 (t, $J=7.1$ Hz, 3H, $C\underline{H}_3CH_2O$–), 0.88 (t, $J=6.8$ Hz, 3H, H_{12}); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.9 (s), 143.3 (s), 141.8 (s), 128.3 (d), 128.2 (d), 128.0 (d), 127.9 (d), 126.8 (d), 126.6 (d), 60.1 (t), 58.0 (d), 54.1 (d), 50.0 (t), 36.9 (t), 33.5 (t), 31.9 (t), 29.7 (t), 29.6 (2t), 29.3 (t), 27.0 (t), 22.7 (t), 19.7 (q), 14.2 (q), 14.1 (q); MS (EI$^+$): *m*/*z* (%) 437 (M^+, 2), 422 (11), 351 (16), 350 (57), 311 (22), 310 (100), 246 (28), 206 (73), 105 (82); HRMS (EI$^+$): calcd for $C_{29}H_{43}NO_2$ 437.3294; found 437.3302; IR (NaCl, cm^{-1}): 3050–2850, 1733.

3.6. Ethyl (3*S*)-3-{benzyl[(1*S*)-1-phenylethyl]amino}-dodecanoate, (*S*,*S*)-13

The spectroscopic data were identical to those for the corresponding (*R*,*R*)-**13** enantiomer. $[\alpha]_D^{20}=-7.7$ (*c* 0.05, $CHCl_3$)

3.7. Methyl (3*R*)-3-{benzyl[(1*R*)-1-phenylethyl]amino}-dodecanoate, (*R*,*R*)-14

A solution of (3*R*)-3-{benzyl-[(*R*)-1-phenylethyl]-amino}dodecanoic acid ethyl ester (*R*,*R*)-**13** (1.66 g, 3.80 mmol) in MeOH (5.0 mL) was added via cannula to a solution of sodium (excess) in MeOH (15.0 mL) at 0°C and the mixture was stirred at rt for 13 h. H_2O was added and the mixture was extracted with EtOAc (3×). The combined organic layers were washed with brine (1×), dried and concentrated to afford, after purification by chromatography (silica gel, hexane/EtOAc 2%), 1.59 g (99%) of a colourless oil. $[\alpha]_D^{20}=+11.9$ (*c* 0.04, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.43–7.20 (m, 10H, 2Ph), 3.85 (q, $J=7.0$ Hz, 1H, $PhCH_3C\underline{H}$–), 3.79 (d, $J=14.8$ Hz, 1H, $PhC\underline{H}_2$–), 3.57 (d, $J=14.8$ Hz, 1H, $PhC\underline{H}_2$–), 3.54 (s, 3H, CH_3O–), 3.29 (m, 1H, H_3), 2.08 (dd, $J=14.5$ and 5.1 Hz, 1H, H_2), 2.03 (dd, $J=14.5$ and 8.0 Hz, 1H, H_2), 1.53 (m, 2H, H_4), 1.35 (d, $J=7.0$ Hz, 3H, $PhC\underline{H}_3CH$–), 1.27 (bs, 14H, H_{5-11}), 0.90 (t, $J=6.8$ Hz, 3H, H_{12}); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.2 (s), 143.2 (s), 141.7 (s), 128.3 (d), 128.2 (d), 128.0 (d), 127.9 (d), 126.8 (d), 126.6 (d), 57.74 (d), 54.1 (d), 51.3 (q), 49.9 (t), 36.7 (t), 33.5 (t), 31.9 (t), 29.61 (t), 29.59 (t), 29.58 (t), 29.3 (t), 27.1 (t), 22.7 (t), 19.3 (q), 14.1 (q); MS (EI$^+$): *m*/*z* (%) 423 (M^+, 1), 408 (11), 351 (12), 350 (41), 297 (15), 296 (78), 246 (20), 192 (68), 105 (100), 91 (69). HRMS (EI$^+$): calcd for $C_{28}H_{41}NO_2$ 423.3137; found 423.3152; IR (NaCl, cm^{-1}): 3050–2850, 1738.

3.8. Methyl (3*S*)-3-{benzyl[(1*S*)-1-phenylethyl]amino}-dodecanoate, (*S*,*S*)-14

The spectroscopic data were identical to those for the corresponding (*R*,*R*)-**14** enantiomer. $[\alpha]_D^{20}=-11.1$ (*c* 0.07, $CHCl_3$).

3.9. (3*R*)-3-Aminododecanoic acid methyl ester, (*R*)-4

To a solution of (3*R*)-3-{benzyl-[(*R*)-1-phenylethyl]-amino}dodecanoic acid methyl ester (*R*,*R*)-**13** (535.0 mg, 1.26 mmol) in acetic acid (10.0 mL) was added 10% palladium on charcoal (64.2 mg) and the mixture was hydrogenated (hydrogen pressure: 60 psi) at 60°C for 17 h. The mixture was filtered through Celite, concentrated and the pH was adjusted to 12 with saturated aqueous $NaHCO_3$. The resulting mixture was extracted with CH_2Cl_2 (3×) and the combined organic layers were dried (Na_2SO_4) and concentrated to afford 284.0 mg (98%) of a brown oil. $[\alpha]_D^{25}=-10.4$ (*c* 0.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.67 (s, 3H, CH_3O–), 3.17 (m, 1H, H_3), 2.46 (dd, $J=15.7$ and 3.7 Hz, 1H, H_2), 2.26 (dd, $J=15.7$ and 8.9 Hz, 1H, H_2), 1.96 (bs, 2H, NH_2), 1.37 (m, 2H, H_4), 1.24 (bs, 14H, H_{5-11}), 0.86 (t, $J=6.8$ Hz, 3H, H_{12}); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.07 (s), 51.5 (q), 48.3 (d), 42.3 (t), 37.6 (t), 31.8 (t), 29.52 (2t), 29.49 (t), 29.3 (t), 26.0 (t), 22.6 (t), 14.1 (q); MS (EI$^+$): *m*/*z* (%) 230 (M^++1, 1), 214 (1.4), 196 (4), 156 (25), 102 (100), 70 (15); HRMS (EI$^+$): calcd for $C_{13}H_{27}NO_2$ 229.2042; found 229.2045; IR (NaCl, cm^{-1}): 3352, 2926, 2855, 1737.

3.10. (3*S*)-3-Aminododecanoic acid methyl ester, (*S*)-4

The spectroscopic data were identical to those for the corresponding (*R*)-**4** enantiomer. $[\alpha]_D^{25}=+7.3$ (*c* 0.22, $CHCl_3$).

3.11. Methyl (3*R*)-3-[(2-{[(1*S*)-1-benzyl-2-methoxy-2-oxoethyl]amino}-2-oxoethyl)amino]dodecanoate, (*S*,*R*)-5

To a solution of amine (*R*)-**5** (250.0 mg, 1.09 mmol) and DIEA (380 μL, 2.18 mmol) in DMF (4.4 mL) was added a solution of the bromide (*S*)-**6** (327.0 mg, 109 mmol) in DMF (10.0 mL) dropwise at 0°C. The reaction mixture was stirred at 25°C for 14 h, H_2O was added and the mixture was extracted with EtOAc (4×). The combined organic layers were washed with brine, dried and concentrated under vacuum. The residue was purified by chromatography (silica gel, hexane/EtOAc 40%) to afford 437.0 mg (89%) of a yellow oil. $[\alpha]_D^{20}=+14.0$ (*c* 0.06, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ

7.82 (d, $J=8.1$ Hz, 1H, Phe NH), 7.30–7.21 (m, 3H, Ph), 7.15 (m, 2H, Ph), 4.85 (m, 1H, H_2), 3.72 (s, 3H, CH_3O–), 3.66 (s, 3H, CH_3O–), 3.29 (d, $J=17.3$ Hz, 1H, $H_{2'}$), 3.21 (d, $J=17.3$ Hz, 1H, $H_{2'}$), 3.19 (dd, $J=13.9$ and 5.8 Hz, 1H, H_3), 3.10 (dd, $J=13.9$ and 7.0 Hz, 1H, H_3), 2.84 (m, 1H, $H_{3''}$), 2.43 (dd, $J=15.5$ and 5.0 Hz, 1H, $H_{2''}$), 2.31 (dd, $J=15.5$ and 7.2 Hz, 1H, $H_{2''}$), 1.65 (s, 1H, NH), 1.30 (m, 2H, $H_{4''}$), 1.25 (m, 15H, $H_{5''}$–$H_{11''}$), 0.89 (t, $J=6.8$ Hz, 3H, $H_{12''}$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.7 (s), 172.0 (s), 171.9 (s), 136.2 (s), 129.2 (d), 128.5 (d), 127.0 (d), 55.1 (d), 52.9 (d), 52.2 (q), 51.6 (q), 49.5 (t), 38.7 (t), 36.8 (t), 34.1 (t), 31.9 (t), 29.6 (t), 29.5 (2t), 29.3 (t), 25.9 (t), 22.7 (t), 14.1 (q); MS (FAB$^+$): m/z (%) 450 (M^++2, 29), 449 (M^++1, 100), 375 (10), 242 (21); HRMS (FAB$^+$): calcd for $C_{25}H_{41}N_2O_5$ 449.3015, found 449.3001; IR (NaCl, cm^{-1}): 3338, 2926, 2854, 1739, 1677.

3.12. Methyl (3*S*)-3-[(2-{[(1*R*)-1-benzyl-2-methoxy-2-oxoethyl]amino}-2-oxoethyl)amino]dodecanoate, (*R*,*S*)-5

The spectroscopic data were identical to those for the corresponding (*S*,*R*)-**5** enantiomer. $[\alpha]_D^{20}=-13.1$ (c 0.08, $CHCl_3$).

3.13. Methyl (3*S*)-3-[(2-{[(1*S*)-1-benzyl-2-methoxy-2-oxoethyl]amino}-2-oxoethyl)amino]dodecanoate, (*S*,*S*)-5

Following the above procedure for the preparation of the dipeptide (*S*,*R*)-**5**, the amine (*S*)-**5** (260.7 mg, 1.14 mmol), DIEA (0.4 mL, 2.27 mmol) and (*S*)-*N*-bromoacetylphenylalanine methyl ester (*S*)-**6** (340.9 mg, 1.14 mmol) in DMF (10.7 mL) were reacted to afford 451.0 mg (88%) of a yellow oil. $[\alpha]_D^{21}=+40.6$ (c 0.06, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.74 (d, $J=8.3$ Hz, 1H, Phe NH), 7.27–7.19 (m, 3H, Ph), 7.12 (m, 2H, Ph), 4.84 (dd, $J=6.7$ and 1.6 Hz, 1H, H_2), 3.71 (s, 3H, CH_3O–), 3.67 (s, 3H, CH_3O–), 3.21 (d, $J=17.2$ Hz, 1H, $H_{2'}$), 3.19 (d, $J=17.5$ Hz, 1H, $H_{2'}$), 3.14 (dd, $J=13.9$ and 5.8 Hz, 1H, H_3), 3.07 (dd, $J=13.9$ and 7.0 Hz, 1H, H_3), 2.78 (m, 1H, $H_{3''}$), 2.36 (dd, $J=15.5$ and 4.9 Hz, 1H, $H_{2''}$), 2.24 (dd, $J=15.4$ and 7.4 Hz, 1H, $H_{2''}$), 1.69 (s, 1H, NH), 1.29 (m, 1H, $H_{4''}$), 1.21 (bs, 15H, $H_{5''}$–$H_{11''}$), 0.85 (t, $J=6.8$ Hz, 3H, $H_{12''}$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.7 (s), 171.9 (s), 171.6 (s), 136.2 (s), 129.2 (d), 128.5 (d), 126.9 (d), 55.0 (d), 52.7 (d), 52.1 (q), 51.6 (q), 49.3 (t), 38.6 (t), 37.9 (t), 34.0 (t), 31.8 (t), 29.5 (2t), 29.4 (t), 29.2 (t), 25.8 (t), 22.6 (t), 14.0 (q); MS (FAB$^+$): m/z (%) 450 (M^++2, 28), 449 (100), 375 (9), 272 (16), 242 (24); HRMS (FAB$^+$): calcd for $C_{25}H_{41}N_2O_5$ 449.3015, found 449.2997; IR (NaCl, cm^{-1}): 3338, 2926, 2854, 1739, 1677.

3.14. Methyl (3*R*)-3-[(2-{[(1*R*)-1-benzyl-2-methoxy-2-oxoethyl]amino}-2-oxoethyl)amino]dodecanoate, (*R*,*R*)-5

The spectroscopic data were identical to those for the corresponding (*S*,*S*)-**5** enantiomer. $[\alpha]_D^{20}=-39.9$ (c 0.07, $CHCl_3$).

3.15. Methyl (3*R*)-3-[(3*S*)-3-benzyl-2,5-dioxopiperazinyl]dodecanoate, (*S*,*R*)-11

A solution of the dipeptide (*S*,*R*)-**5** (12.9 mg, 0.029 mmol) in toluene (0.5 mL) was stirred for 5 days at 130°C in a Schlenk tube. The reaction mixture was evaporated and the residue purified by HPLC (column: Spherisorb S5 NH2, 10×250 mm, hexane/*i*-PrOH 10%, 2.5 mL/min, $t_R=15.4$ min) to afford 2.4 mg (40%) of a white solid and 6.4 mg of starting material as a yellow oil. $[\alpha]_D^{25}=-37.6$ (c 0.71, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.33–7.19 (m, 5H, Ph), 6.08 (bs, 1H, NH), 4.67 (m, 1H, $H_{3''}$), 4.17 (m, 1H, H_2), 3.66 (d, $J=17.2$ Hz, 1H, $H_{2'}$), 3.65 (s, 3H, CH_3O–), 3.41 (d, $J=17.2$ Hz, 1H, $H_{2'}$), 3.22 (dd, $J=13.7$ and 3.6 Hz, 1H, H_3), 2.95 (dd, $J=13.7$ and 8.2 Hz, 1H, H_3), 2.50 (dd, $J=15.1$ and 6.2 Hz, 1H, $H_{2''}$), 2.37 (dd, $J=15.1$ and 6.2 Hz, 1H, $H_{2''}$), 1.55–1.49 (m, 2H, $H_{4''}$), 1.22 (bs, 14H, $H_{5''}$–$H_{11''}$), 0.85 (t, $J=6.7$ Hz, 3H, $H_{12''}$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.1 (s), 165.8 (s), 165.7 (s), 135.4 (s), 129.6 (d), 129.0 (d), 127.6 (d), 57.0 (d), 51.9 (q), 51.4 (d), 45.0 (t), 40.4 (t), 36.7 (t), 31.8 (t), 31.0 (t), 29.5 (t), 29.4 (t), 29.2 (t), 29.1 (t), 26.0 (t), 22.6 (t), 14.1 (q); MS (FAB$^+$): m/z (%) 418 (27), 417 (M^++1, 100), 416 (12), 289 (14); HRMS (FAB$^+$): calcd for $C_{24}H_{37}N_2O_4$ 417.2753; found 417.2734; IR (NaCl, cm^{-1}): 2924, 1735, 1652.

3.16. Methyl (3*S*)-3-[(3*R*)-3-benzyl-2,5-dioxopiperazinyl]dodecanoate, (*R*,*S*)-11

The spectroscopic data were identical to those for the corresponding (*S*,*R*)-**11** enantiomer. $[\alpha]_D^{26}=+37.1$ (c 0.76, $CHCl_3$).

3.17. Methyl (3*S*)-3-[(3*S*)-3-benzyl-2,5-dioxopiperazinyl]dodecanoate, (*S*,*S*)-11

Following the above procedure for the preparation of dioxopiperazine (*S*,*R*)-**11**, the dipeptide (*S*,*S*)-**5** (105.6 mg, 0.235 mmol) in toluene (5.0 mL) was reacted to afford, after purification by chromatography (silica gel, hexane/EtOAc 50% to EtOAc), 47.1 mg (66%) of a white solid and 28.9 mg of starting material as a yellow oil. $[\alpha]_D^{20}=-68.8$ (c 0.43, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.35–7.21 (m, 5H, Ph), 6.25 (bs, 1H, NH), 4.67 (m, 1H, $H_{3''}$), 4.23 (dd, $J=6.5$ and 3.6 Hz, 1H, H_2), 3.70 (d, $J=17.3$ Hz, 1H, $H_{2'}$), 3.66 (s, 3H, CH_3O–), 3.37 (bd, $J=17.2$ Hz, 1H, $H_{2'}$), 3.25 (dd, $J=13.8$ and 3.2 Hz, 1H, H_3), 3.02 (dd, $J=13.8$ and 7.8 Hz, 1H, H_3), 2.58–2.47 (m, 2H, $H_{2''}$), 1.55–1.43 (m, 2H, $H_{4''}$), 1.27 (bs, 14H, $H_{5''}$–$H_{11''}$), 0.89 (t, $J=6.8$ Hz, 3H, $H_{12''}$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.1 (s), 166.0 (s), 165.7 (s), 135.3 (s), 129.7 (d), 129.0 (d), 127.5 (d), 56.6 (d), 51.9 (q), 51.7 (d), 45.3 (t), 40.0 (t), 36.4 (t), 31.8 (t), 31.3 (t), 29.5 (t), 29.4 (t), 29.3 (t), 29.2 (t), 26.1 (t), 22.7 (t), 14.1 (q); MS (FAB$^+$): m/z (%) 418 (27), 417 (M^++1, 100), 416 (13), 205 (9); HRMS (FAB$^+$): calcd for $C_{24}H_{37}N_2O_4$ 417.2753; found 417.2738; IR (NaCl, cm^{-1}): 2924, 1735, 1652.

3.18. Methyl (3*R*)-3-[(3*R*)-3-benzyl-2,5-dioxopiperazinyl]dodecanoate, (*R*,*R*)-11

The spectroscopic data were identical to those for the corresponding (*S*,*S*)-**11** enantiomer. $[\alpha]_D^{26}=+67.2$ (c 0.54, $CHCl_3$).

3.19. Methyl 3-({(3*R*)-3-[(3*S*)-3-benzyl-2,5-dioxopiperazinyl]dodecanoyl}amino)propylcarbamate, (*S*,*R*)-2

A mixture of the dioxopiperazine (*S*,*R*)-**11** (7.6 mg, 0.180 mmol) and 1,3-diaminopropane (0.5 mL, 6.0 mmol) was stirred at 25°C for 15 h and the excess 1,3-diaminopropane was removed under vacuum. The residue was dissolved in CH_2Cl_2 (0.75 mL) and acetyl chloride (1.3 μL, 0.018 mmol) and Et_3N (2.5 μL, 0.018 mmol) were added and the mixture was stirred at 25°C for 30 min. To the reaction mixture was added H_2O and the organic material was extracted with CH_2Cl_2 (4×). The combined organic layers were dried (Na_2SO_4) and concentrated. Purification by HPLC (Spherisorb S5 NH2, 10×250 mm, CH_2Cl_2/MeOH 5%, 2.5 mL, t_R = 13.8 min) yielded 6.1 mg (67%) of a colourless oil. $[\alpha]_D^{21} = -53.1$ (*c* 0.06, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.36–7.20 (m, 5H, Ph), 6.42 (bs, 1H, NH), 6.18 (bs, 1H, NH), 5.74 (s, 1H, Phe NH), 4.56 (m, 1H, $H_{3''}$), 4.16 (m, 1H, H_2), 3.73 (d, J = 17.8 Hz, 1H, $H_{2'}$), 3.66 (d, J = 17.4 Hz, 1H, $H_{2'}$), 3.31–3.23 (m, 5H, $H_{1'''}$+$H_{3'''}$+H_3), 2.95 (dd, J = 13.8 and 9.2 Hz, 1H, H_3), 2.49 (m, 1H, $H_{2''}$), 2.32 (dd, J = 14.7 and 5.3 Hz, 1H, $H_{2''}$), 2.00 (s, 3H, Ac), 1.65–1.59 (m, 2H, $H_{2'''}$), 1.25 (bs, 16H, $H_{4''}$–$H_{11''}$), 0.88 (t, J = 7.0 Hz, 3H, $H_{12''}$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.9 (s), 170.5 (s), 166.5 (s), 166.1 (s), 135.4 (s), 129.8 (d), 129.1 (d), 127.6 (d), 57.1 (d), 53.4 (d), 46.0 (t), 40.4 (t), 39.4 (t), 36.3 (2t), 32.2 (t), 31.6 (t), 29.6 (t), 29.5 (t), 29.5 (t), 29.3 (2t), 26.3 (t), 23.4 (q), 22.7 (t), 14.2 (q); MS (FAB$^+$): *m*/*z* (%) 523 (15), 502 (31), 501 (M^++1, 100), 500 (12), 499 (8), 385 (21), 343 (12), 315 (9); HRMS (FAB$^+$): calcd for $C_{28}H_{45}N_4O_4$ 501.3441; found 501.3449.

3.20. Methyl 3-({(3*S*)-3-[(3*R*)-3-benzyl-2,5-dioxopiperazinyl]dodecanoyl}amino)propylcarbamate, (*R*,*S*)-2

The spectroscopic data were identical to those for the corresponding (*S*,*R*)-**2** enantiomer. $[\alpha]_D^{21} = +53.5$ (*c* 0.11, $CHCl_3$).

3.21. Methyl 3-({(3*S*)-3-[(3*S*)-3-benzyl-2,5-dioxopiperazinyl]dodecanoyl}amino)propylcarbamate, (*S*,*S*)-2

A mixture of the dioxopiperazine (*S*,*S*)-**11** (1.2 mg, 0.003 mmol) and 1,3-diaminopropane (0.2 mL) was stirred at 25°C for 17 h and the excess 1,3-diaminopropane was removed under vacuum. Ac_2O (0.2 mL, 2.39 mmol) and Et_3N (0.2 mL, 1.43 mmol) were added and the mixture was stirred for 1 h. To the resulting solution was added H_2O (0.5 mL) and the organic material was extracted with EtOAc (4×). The combined organic layers were washed with 0.5 M aqueous HCl (1×), 2 M aqueous K_2CO_3 (1×) and saturated aqueous $CaCl_2$ (1×), dried (Na_2SO_4) and concentrated. Purification by HPLC (μPorasil column, 10 μm 7.8×300 mm, EtOAc/MeOH 11%, 2.0 mL/min, t_R = 12.1 min) afforded 0.4 mg (28%) of a colourless oil. $[\alpha]_D^{19} = -19.4$ (*c* 0.07, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.49–7.18 (m, 5H, Ph), 6.38 (bs, 1H, NH), 6.02 (bs, 1H, Phe NH), 5.22 (m, 1H, H_2), 4.60 (m, 1H, $H_{3''}$), 3.59 (d, J = 18.1 Hz, 1H, $H_{2'}$), 3.29–3.13 (m, 5H, $H_{1'''}$+$H_{3'''}$+H_3), 3.06 (m, 1H, H_3), 2.67 (d, J = 18.1 Hz, 1H, $H_{2'}$), 2.40–2.26 (m, 2H, $H_{2''}$), 1.96 (s, 3H, Ac), 1.52 (bs, 1H, $H_{2'''}$), 1.26 (bs, 16H, $H_{4''}$–$H_{11''}$), 0.87 (t, J = 6.7 Hz, 3H, $H_{12''}$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.8 (s), 170.4 (s), 166.3 (s), 166.2 (s), 135.2 (s), 129.9 (d), 128.8 (d), 127.4 (d), 56.7 (d), 51.6 (d), 44.5 (t), 39.9 (t), 38.7 (t), 36.3 (2t), 31.8 (t), 31.4 (t), 29.6 (t), 29.5 (t), 29.4 (t), 29.2 (2t), 26.1 (t), 23.2 (q), 22.6 (t), 14.1 (q); MS (FAB$^+$): *m*/*z* (%) 523 (15), 502 (31), 501 (M^++1, 100), 500 (13), 499 (9), 385 (22), 343 (15), 315 (10); HRMS (FAB$^+$): calcd for $C_{28}H_{45}N_4O_4$ 501.3441; found 501.3446.

3.22. Methyl 3-({(3*S*)-3-[(3*S*)-3-benzyl-2,5-dioxopiperazinyl]dodecanoyl}amino)propylcarbamate, (*S*,*S*)-2

The spectroscopic data were identical to those for the corresponding (*S*,*S*)-**2** enantiomer. $[\alpha]_D^{19} = +16.6$ (*c* 0.07, $CHCl_3$).

3.23. *nat*-2

To a solution of a fraction of *Didemnum rodriguesi* containing Etzionin **1** as the major metabolite (16.0 mg, 0.034 mmol) and 20% Na_2CO_3 (0.5 mL) in MeOH (0.5 mL) was added slowly $TiCl_3$ (165.0 mg, 20% aqueous solution, 0.016 mmol), keeping the pH at 7 by adding 20% aqueous Na_2CO_3. The mixture was stirred at rt for 4 h and H_2O was added. The organic material was extracted with EtOAc (3×) and the combined organic layers were dried (Na_2SO_4) and concentrated. Acetic anhydride (0.2 mL, 2.11 mmol) and pyridine (0.2 mL, 2.47 mmol) were added and the mixture was stirred at rt for 1 h. The aqueous phase was extracted with EtOAc (3×) and the combined organic layers were dried (Na_2SO_4) and concentrated. Purification by HPLC (μPorasil column 10 μm, 7.8×300 mm, EtOAc/MeOH 11%, 2.0 mL/min, t_R = 16.9 min) yielded 6.5 mg (42%) of a yellow oil. $[\alpha]_D^{23} = -51.9$ (*c* 0.08, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.37–7.18 (m, 5H, Ph), 6.52 (bs, 1H, NH), 6.24 (bs, 1H, NH), 5.90 (s, 1H, Phe NH), 4.50 (m, 1H, $H_{3''}$), 4.15 (bm, 1H, H_2), 3.70–3.56 (m, 2H, $H_{2'}$), 3.28–3.16 (m, 5H, $H_{1'''}$+$H_{3'''}$+H_3), 2.96 (m, 1H, H_3), 2.49 (m, 1H, $H_{2''}$), 2.30 (dd, J = 14.4 and 5.2 Hz, 1H, $H_{2''}$), 1.97 (s, 3H, Ac), 1.49–1.38 (m, 2H, $H_{2'''}$), 1.22 (bs, 16H, $H_{4''}$–$H_{11''}$), 0.85 (t, J = 7.0 Hz, 3H, $H_{12''}$).

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