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Synthesis of 2-Amino-8-oxodecanoic Acids (Aodas) Present in Natural Hystone Deacetylase Inhibitors

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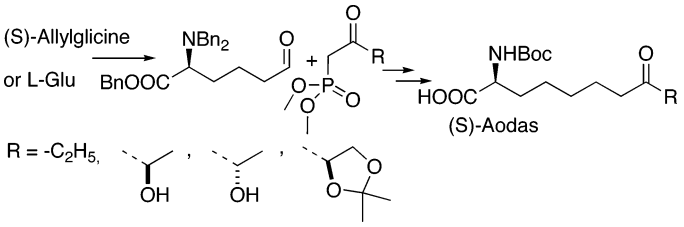

Differently substituted 2-amino-8-oxodecanoic acids (Aodas), present in naturally occurring inhibitors of hystone deacetylase (HDAC), have been prepared using a convergent approach. The configuration in position 2 was derived from enantiomerically pure allylglycine or glutamic acid, whereas the stereochemistry of the substituent in position 9 derived from lactic acid or glyceraldehyde derivatives. Starting from allylglycine, (*S*)-Aodas, protected at the nitrogen as Boc or Fmoc, were obtained in four steps in about 30% overall yield. These products have been used to prepare a simplified analogue of a natural cyclic tetrapeptide HDAC inhibitor by SPPS.

Introduction

The organization of DNA into chromatin has important implications in gene expression. DNA methylation and hystone protein modification influence the effective activity of a gene that, although present in the genome, may be silenced or expressed by this epigenetic mechanism.[1] Hystone acetylation results in activation of genes increasing accessibility of nucleosome DNA, whereas hystone deacetylation may silence key genes during differentiation.[2] Several reports associate epigenetic alterations with cancer development and, consequently, many molecules have been evaluated to restore the initial equilibrium in the so-called "epigenetic therapy".[3] As hystone deacetylase (HDAC) is a class of enzymes implicated in epigenetic disorders, HDAC inhibitors have been shown to induce growth arrest,[4] terminal differentiation,[5] apoptosis,[6] and antiangiogenesis.[7] An important class of HDAC inhibitors is constituted by cyclic tetrapeptides of natural origin.[8] With the exception of a few examples, they are essentially constituted by a large cap group containing hydrophobic amino acids and a lateral side chain

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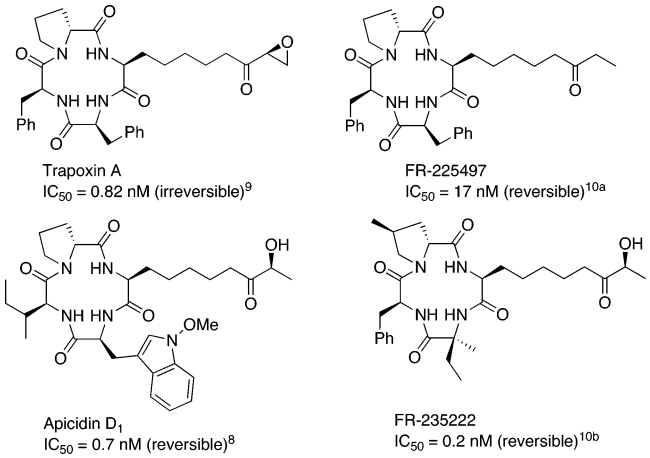


FIGURE 1. Natural HDAC inhibitor tetrapeptides.

formed by 2-amino-8-oxa-decanoic acid (Aoda) and its derivatives, ending with a chelating group for the zinc present in the active site of the enzyme.

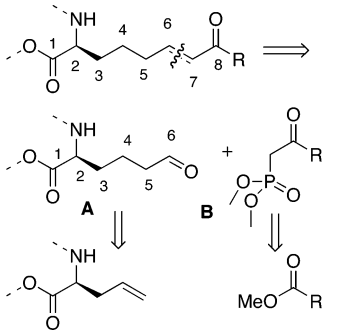
All members of the Aoda family bear a carbonyl in position 8 and additional moieties relevant for protein binding in position 9 or 10 (such as OH or an epoxide). The nature of this additional binding site influences the reversibility of the inhibition. Epoxide-containing molecules (such as trapoxins) are irreversible inhibitors,[9] whereas alkyl- or OH-containing Aoda tetrapeptides behave as reversible inhibitors (see Figure 1).[10] Despite the importance of these noncoded amino acids, relatively few syntheses of these molecules have been reported[11] and are mainly of the parent compound with no substituents on the last two carbons.[12]

Results and Discussion

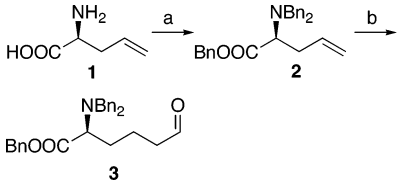
Following our interest in the synthesis of biologically relevant α-amino acids,[13] we wanted to develop a general stereocontrolled synthesis of all possible 2-amino-8-oxodecanoic acids present in nature and eventually of other synthetic analogues for the discovery of new and more selective HDAC inhibitors. A possible retrosynthesis is reported in Scheme 1, where a disconnection at the C6−C7 bond is proposed.

The corresponding aldehyde **A** could be prepared starting from readily available natural amino acids and the phosphonates **B** could be obtained from the corresponding esters. This

SCHEME 1



SCHEME 2[a]



[a] Reagents and conditions: (a) BnBr, Na_2CO_3, NaOH, H_2O, reflux, 1 h; (b) $HRh(CO)(PPh_3)_3$ Xantphos (10% of a 1:2 mixture) H_2/CO 1:1, 20 atm, 40 °C for 96 h.

synthesis allows the introduction of different moieties (enantiomerically pure, if required) at the position adjacent to the carbonyl in order to modulate their inhibitory activity. The most straightforward approach toward aldehyde **A** seemed to be the hydroformylation of (*S*)-allylglycine.[14] As the last step of the proposed synthetic scheme was hydrogenation, we decided to protect allylglycine as the tribenzyl derivative, to get their simultaneous deprotection during the reduction of the double bond between C6−C7. Thus, (*S*)-allylglycine was protected with benzyl bromide, following the procedure developed by Reetz,[15] giving compound **2** in 90% yield (Scheme 2). Hydroformylation of **2** was carried out using the catalytic system $HRh(CO)(PPh_3)_3$ Xantphos[16] under 20 atm of H_2/CO 1:1 at 40 °C for 96 h, and compound **3** was isolated in 75% yield as a single regioisomer (^{1}HNMR 400 MHz analysis). The combined use of the hindered protection at the nitrogen and the use of the ligand Xantphos prevented the formation of the branched isomer.

An alternate (longer) synthesis of aldehyde **3**, designed for laboratories not equipped with autoclaves and security systems for handling CO, started from L-glutamic acid, which was fully benzylated using the same procedure applied to allylglycine. The tetrabenzyl derivative was reduced with DIBAL in order to get the alcohol **5** (3 equiv of DIBAL at 0 °C), which was then oxidized to aldehyde **6** under standard Swern conditions.[17] Homologation with methoxymethyltriphenyl phosphonium chlo-

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(13) For the last paper of the series, see: Esposito, A.; Piras, P. P.; Ramazzotti, D.; Taddei, M. *Org. Lett*. **2001**, *3*, 3273.

(14) Enantiomerically pure allylglicine is commercialy available and can be obtained following different procedures as, for example: Wolf, L.; Sonke, T.; Tjen, K. C. M. F.; Kaptein, B.; Broxterman, Q. B.; Schoemaker, H. E.; Rutjes, F. P. J. T. *Adv. Synth. Catal*. **2001**, *343*, 662 and references therein.

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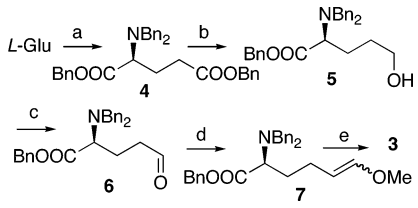
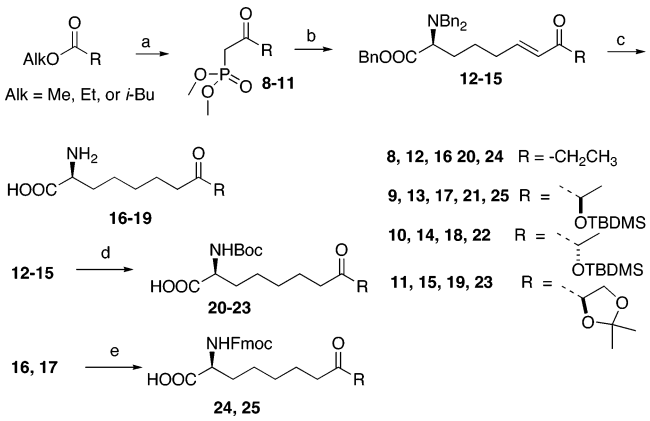
SCHEME 3[a]



[a] Reagents and conditions: (a) BnBr, Na_2CO_3, NaOH, H_2O, reflux, 1 h; (b) DIBAL, THF, 0 °C; (c) $(COCl)_2$, DMSO, Et_3N in CH_2Cl_2, −78 °C, 30 min; (d) $MeOCH_2PPh_3Cl$, $LiN(SiMe_3)_2$, THF, 0 °C then 12 h rt; (e) HCl 6N EtOAc, 20 min, rt.

SCHEME 4[a]



[a] Reagents and conditions: (a) $CH_3PO(OMe)_2$, BuLi, THF, −78 °C, 1 h; (b) **3**, LiCl, DIPEA, MeCN, rt, 72 h; (c) H_2 (6 atm), $Pd(OH)_2$ on C (10%) MeOH, rt, 12 h; (d) H_2 (6 atm.), $Pd(OH)_2$ on C (10%) Boc_2O (1.5 equiv), MeOH, rt, 12 h; (e) FmocOSu, Na_2CO_3, acetone/H_2O 1/1, rt, 24 h.

ride and $LiN(SiMe_3)_2$ in THF, followed by acidic workup, gave the required aldehyde **3** (Scheme 3). All steps of the process were optimized, and aldehyde **3** was obtained in 75% overall yield on a multigram scale.

At the same time, the β-ketophosphonates **8**−**11** were obtained from the corresponding commercially available esters and dimethyl methylphoshonate in the presence of BuLi in THF at −78 °C. For the synthesis of the two epimers of 2-amino-9-hydroxy-8-oxadecanoic acids (**17** and **18**), commercially available (*S*)-methyl lactate and (*R*)-isobutyl lactate were employed as the starting esters after protection of the OH as OTBDMS (Scheme 4). For the synthesis of **19** (precursor of epoxide-containing tetrapeptides), commercially available (*R*)-methyl-2,2-dimethyl-1,3-dioxolane-4-carboxylate was employed. Phosphonates **9** and **10** were sufficiently stable to be isolated, purified by column chromatography, and fully characterized, whereas compounds **8** and **11** showed low stability and had to be used in the next step without purification. Wittig−Horner−Emmons reaction of phosphonates **8**−**11** with aldehyde **3** gave the unsaturated ketones **12**−**15** (Scheme 4). The reaction was carried out under strictly anhydrous conditions in MeCN using LiCl and DIPEA as base. After stirring at room temperature for 72 h, the required compounds were obtained in yields higher than 80% after column chromatography (see Experimental Section). The last step (hydrogenation and hydrogenolysis of the protective groups) was crucial for the success of the synthesis. The use of Pd/C did not succeed in deprotection of the benzyl groups (exclusively reduction of the double bond occurred). Moreover, working at low H_2 pressure or reducing reaction times gave the *N*-monobenzyl amino acid. The reaction was carried out by hydrogenation using $Pd(OH)_2$/C in dry MeOH at 6 atm for 12 h (Parr apparatus). After catalyst removal by filtration on Celite and purification through a small silica plug, compounds **16**−**19** were obtained in 70−80% yields. Using these conditions, the other protections on the side chain remained untouched. In the case of compounds **13** and **14**, when $CHCl_3$ was used as the solvent, the simultaneous deprotection of the OTBDMS group was observed. When the hydrogenation was carried out in the presence of Boc_2O (1.5 equiv), the corresponding *N*-Boc-protected amino acids **20**−**23** were obtained in a single step from the tribenzyl derivatives. On the other hand, the introduction of the Fmoc protection was possible by reaction of the free amino acid with FmocOSu in aqueous Na_2CO_3. Compounds **16** and **17** were transformed into the Fmoc-protected derivatives **24** and **25** in high yields. These products, like **20**−**23**, are suitable for use in SPPS.

HPLC analysis and high field ^{1}H NMR spectra of compounds **21**, **22**, and **23** showed the presence of a single diastereoisomer, confirming that the stereochemical integrity was maintained. As expected, (*R*)- and (*S*)-Aodas gave rise to subtle differences in their pattern of NMR signals (in both ^{1}H and ^{13}C NMR spectra), very likely owing to the remote character of their two stereogenic centers separated by a flexible six-carbon chain. However, a more sensible spectroscopic difference stemmed from the characteristic pattern of doubled signals contained in the NMR spectra of (*R*)-Aoda, originating from the slow interconversion (on the NMR time scale) of rotamers with different Boc spatial orientation.

Compound **21** was employed for the synthesis of a new synthetic tetrapeptide by SPSS starting with a 2-chlorotrityl resin.[18] D-Pro was loaded on the resin followed by HATU/HOBT promoted couplings with L-Phe and L-Aib. Product **21** (*N*-Boc-OTBDMS-Ahoda) was introduced as the last amino acid before cleavage from the resin with AcOH/TFE/TIS that gave the linear precursor **27** in 70% yield.

After cleavage and removal of TBDMS and Boc groups from Ahoda by treatment with TFA-H_2O-TIS (95% yield),[19] the unprotected linear tetrapeptide **28** was cyclized by exposure to HATU (2 equiv) and DIEA (2 equiv) in a very diluted solution (7.7×10^{-5} M in DCM) to avoid the side formation of the cyclodimeric sequence of **29** as byproduct (Scheme 5). The cyclized product **29** was obtained in 68% yield after HPLC purification and was characterized by ES-MS and ^{1}H and ^{13}C NMR spectroscopic data. Compound **29** has been tested gainst class III HDACs (experiments carried out on rat smooth muscle cell line A7r5), showing an IC_{50} of 10 mM.[20] Athough the activity of this product was not high as some of the natural tetrapeptides, we have demonstrated that it is possible to prepare simplified HDAC inhibitor tetrapeptides with the incorporation of the Aoda moiety.

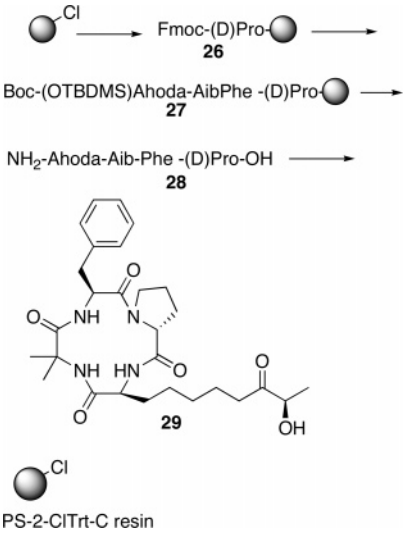
In conclusion, we have developed a general efficient method for the synthesis of all members of the Aoda family present in nature and their synthetic analogues. The final products are

(18) Terracciano, S.; Bruno, I.; Bifulco, G.; Copper, J. E.; Smith, C. D.; Gomez-Paloma, L.; Riccio, R. *J. Nat. Prod.* **2004**, *67*, 1325−1331. Napolitano, A.; Rodriquez, M.; Bruno, I.; Marzocco, S.; Autore, G.; Riccio, R.; Gomez-Paloma, L. *Tetrahedron* **2003**, *59*, 10203−10211.

(19) The amount of TIS used as carbocation scavenger had to be carefully adjusted because of its ability to promote a reductive amination (as side reaction) between the free amino terminus and the Ahoda carbonyl group in position 8, giving rise to an eight-membered ring.

(20) Govin, J.; Caron, C.; Rousseaux, S.; Khochbin, S. *Trends Biochem. Sci.* **2005**, *30*, 357.

SCHEME 5



obtained in four steps, starting from (*S*)-allylgycine or in six steps from L-Glu. The synthesis is general and may be used to introduce different functional groups at the R position, just starting from different esters. Moreover, these compounds are ready for the introduction in a tetrapeptide for further cyclization and synthesis of new compounds related to apicidin and trapoxin families.[21]

Experimental Section

(*S*)-2-Dibenzylamino-6-oxo-hexanoic Acid Benzyl Ester (3) from Tribenzylallylglycine. A solution containing compound **2** (1.0 g, 2.6 mmol), $HRh(CO)(PPh_3)_3$ (47.6 mg, 0.05 mmol) and Xanthphos (0.168 g, 0.306 mmol) in dry toluene (20 mL) was inserted into a 150 mL stainless steel autoclave, closed under nitrogen, and pressurized with 20 atm of CO/H_2 1:1. The mixture was stirred for 96 h at 40 °C. After cooling, the autoclave was degassed and the solvent was evaporated under vacuum. The crude was dissolved in diethyl ether and passed through a short path of silica gel to recover **3** (0.78 g, 75% yield) sufficiently pure for the next step. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.60−1.80 (m, 4H), 2.11−2.19 (m, 2H), 3.23 (X part of an ABX system, 1H), 3.41 (d, J = 8 Hz, 2H), 3.80 (d, J = 8 Hz, 2H), 5.12 (AB system, 2H), 7.15−7.40 (m, 15H), 9.57 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 18.6, 28.9, 42.2, 54.4 (2C), 60.2, 66.1, 125.2 (2C), 125.3, 126.3 (4C), 126.5 (2C), 128.6 (4C), 128.9 (2C), 136.2 (2C), 139.2, 172.1, 202.1. IR (neat, cm^{-1}) ν 3090, 2870, 2800, 1740, 1730, 1550. MS-(ES) $C_{27}H_{29}NO_3$ *m/z* 416 $(M + H)^+$.

(*S*)-2-Dibenzylamino-6-oxo-hexanoic Acid Benzyl Ester (3) from Aldehyde 6. Methoxymethyltriphenylphosphomium chloride (0.771 g, 2.25 mmol) was dissolved in dry THF (8 mL), and to this solution, cooled to 0 °C, was slowly added $LiN(SiMe_3)_2$ (2.4 mL of a 1 M solution in THF). After 1 h at 0 °C, aldehyde **6**[17a] (0.597 g, 1.5 mmol) in dry THF (8 mL) was added. The mixture was stirred overnight at room temperature. Water was added and the two phases separated. The aqueous layer was washed several times with Et_2O, and the organic fractions were collected, dried, and evaporated. The crude was purified using the Sepacore system (silica gel column, petroleum ether 60−80/EtOAc 3:1) to give crude compound **22** (0.48 g, 75% yield). This product was dissolved in EtOAc (8 mL) followed by a solution of 6 N HCl (4 mL).[22] The mixture was stirred at room temperature for 20 min, and then a saturated solution of Na_2CO_3 added. The organic phase was separated, and the aqueous layer was washed several times with EtOAc. The organic fractions were collected and dried, and the solvent was evaporated to give compound **3** (0.436 g. 94% yield). The spectra of the product were identical to the product obtained by hydroformilation.

(2*S*,9*S*,*E*)-2-Dibenzylamino-9-(*tert*-butyldimethylsilyloxy)-8-oxo-6-decenoic Acid Benzyl Ester 14, General Procedure. To a solution of phosphonate **10** (0.293 g, 0.94 mmol) in dry MeCN (5 mL) was added dry LiCl (42.7 mg, 0.94 mmol), followed by freshly distilled DIPEA (98.0 mg, 0.78 mmol). After stirring for 2 h at room temperature, aldehyde **3** (0.300 g, 0.72 mmol) in MeCN was added and the mixture was stirred at room temperature for 72 h. A saturated solution of NaCl was used for the quench and the organic layer was separed. The aqueous phase was extracted several times with EtOAc, and all the organic fractions were collected, dried, and purified using a Sepacore system (silica gel column, petroleum ether 60−80/ EtOAc 6:1) to give compound **14** (0.37 g, 87% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.04 (s, 3H), 0.06 (s, 3H), 0.89 (s, 9H), 1.28 (d, J = 7 Hz, 3H), 1.35−1.82 (m, 4H), 1.87−2.01 (m, 2H), 3.24 (X part of an ABX system, 1H), 3.49 (d, J = 8 Hz, 2H), 3.88 (d, J = 8 Hz, 2H), 4.21 (q, J = 7 Hz, 1H), 5.18 (AB system, 2H), 6.58 (d, J = 13 Hz, 1H), 6.89 (dt, ^AJ = 13 Hz, ^BJ = 8 Hz, 1H), 7.20−7.40 (m, 15H). ^{13}C NMR (50 MHz, $CDCl_3$): δ −4.9, −4.8, 18.1, 21.1, 24.5, 25.7(3C), 28.9, 32.1, 54.4(2C), 60.2, 66.0, 74.4, 124.2, 127.0(2C), 128.2(4C), 128.3(2C), 128.4(4C), 128.5-(2C), 128.8(2C), 136.1, 139.4, 148.2, 172.0, 201.7. IR (neat, cm^{-1}) ν 3070, 1740, 1705. MS (ES) *m/z* 600 $(M + H)^+$. Anal. Calcd for $C_{37}H_{49}NO_4Si$: C, 74.08; H, 8.23; N, 2.23. Found: C, 73.87; H, 8.20; N, 2.22.

(2*S*,9*S*)-2-(*tert*-Butoxycarbonylamino)-9-(*tert*-butyldimethylsilyloxy)-8-oxodecanoic Acid 21, General Procedure. $Pd(OH)_2$ on C (20 mg) was dissolved in dry MeOH (4 mL) and inserted into the bottle connected with a Parr apparatus. After 2 cycles of vacuum-nitrogen, ketone **14** (0.200 g, 0.334 mmol), dissolved in dry MeOH (2 mL), was added, followed by Boc_2O (0.145 g, 0.66 mmol). The bottle was filled with H_2 at 6 atm and shaken at room temperature for 12 h. The bottle was degassed, and the catalyst was filtered (*attention: the Pd residue may be pyrophoric*) and washed several times with MeOH. The solvent was evaporated and product **21** was purified using the Sepacore system (silica gel column, $CHCl_3$/MeOH 98:2). Obtained 0.108 g, 75% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.08 (s, 6H), 0.87 (s, 9H), 1.21 (d, J = 7 Hz, 3H), 1.45 (s, 9H), 1.25−1.87 (m, 8H), 2.49−2.59 (m, 2H), 4.12 (q, J = 7 Hz, 1H), 4.20−4.31 (m, 1H), 4.95−5,05 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ −3.7, −3.5, 18.1, 21.1(3C), 24.3(3C), 25.0, 25.7, 28.9, 29.2, 33.4, 38.4, 57.6, 79.6, 80.6, 158.2, 172.5, 215.2. IR (neat, cm^{-1}) ν 3250, 3100−2660 (broad), 1740, 1730, 1725. MS (ES) $C_{21}H_{41}NO_6$ *m/z* 454 $(M + Na)^+$. Anal. Calcd for : C, 58.43; H, 9.57; N, 3.25. Found: C, 58.37; H, 9.54; N, 3.22.

Cyclo-(2*S*,9*R*)-Ahoda-Aib-L-Phe-D-Pro (29). The tetrapeptide wa prepared using classical procedures for solid-phase synthesis and cleaved from the resin as reported in Supported Information. The cyclization step was performed in solution at a concentration of 7.7×10^{-5} M with HATU (18.8 mg, 0.05 mmol) and DIEA (10.9 μL, 0.062 mmol) in DCM. The solution was stirred at 4 °C for 1 h and then allowed to warm to room temperature for 1 h. The solvent was removed under reduced pressure. The crude cyclopeptide (36.9 mg) was purified by semipreparative RP HPLC using the following gradient: from 5% B to 100% B over 30 min at flow rate of 4 mL/min. The binary solvent system (A/B) was as

(21) For the preparation of the epoxide, the acetonide must be deprotected and cyclized as described in: Chow, S.; Kitching W *Tetrahedron: Asymmetry* **2002**, *13*, 779.

(22) One of the referees remarked on the possibility of reaction between AcOEt and 1 N HCl. In all cases investigated, we never experienced this problem. However, changing the solvent from AcOEt to THF, Et_2O, or acetone gave the required aldehyde in much lower yield.

follows: 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B). The absorbance was detected at 240 nm. The HPLC analysis showed one main peak $t_R = 19.20$ min that was identified as pure **29** (63% in yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ: **Aoh** 4.16 (H-2, m, 1H), 1.80 (H-3a, m, 1H,), 1.60 (H-3b, m, 1H,), 1.30 (H_2-4, m, 2H,), 1.31 (H_2-5, m, 2H,), 1.61 (H_2-6, m, 2H,), 2.50 (H-7a, m, 1H), 2.42 (H-7b, m, 1H), 4.25 (H-9, m, 1H,), 1.38 (H_3-10, d, J = 7 Hz, 3H); **Pro** 4.65 (H-13, d, J = 8 Hz, 1H) 2.32 (H-14, m, 1H) 2.12 (H-14, m, 1H) 2.52 (H_2-15, m, 2H) 3.85 (H-16a, m, 1H) 2.47 (H-16b, m, 1H); **Phe** 5.15 (H-19, ddd, J = 9.5, 9.5, 6 Hz, 1H) 3.22 (H-20a, dd, J = 13.5, 9.5 Hz, 1H) 2.98 (H-20b, dd, J = 13.5, 6 Hz, 1H) 7.22 (H-22/H-26, m, 2H) 7.25 (H-23/H-25, m, 2H) 7.20 (H-24, t, J = 8.0 Hz 1H); **Aib** 1.8 (H_3-30, s, 3H) 1.35 (H_3-31, s, 3H). Exchangeable protons not detectable due to low sample concentration. ES HRMS calcd for $C_{28}H_{41}N_4O_6$ (M^+) 529.3026, obsd 529.3049.

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Supporting Information Available: Procedures for the preparation of compounds **2**, **8**−**11**, **16**−**19**, **24**−**25**, and **29** and characterization of compounds **12**, **13**, **15**−**20**, **22**−**25**, and **29**. This material is available free of charge via the Internet at http://pubs.acs.org.

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