Tetrahedron Vol 45 No 16 pp 5039 to 5050 1989
Printed in Great Britain

0040-4020/89 $3 00+ 00

ISOPROPENYL CHLOROCARBONATE (IPCC)[1] IN AMINO ACID AND PEPTIDE CHEMISTRY: ESTERIFICATION OF N-PROTECTED AMINO ACIDS; APPLICATION TO THE SYNTHESIS OF THE DEPSIPEPTIDE VALINOMYCIN

Choukri Zeggaf, Joël Poncet, Patrick Jouin*, Marie-Noëlle Dufour, and Bertrand Castro

Centre CNRS-INSERM de Pharmacologie-Endocrinologie, 34094 Montpellier-cedex, France

(*Received in Belgium* 8 *May* 1989)

Summary Esterification of N-protected α-amino acids was achieved via isopropenyl chlorocarbonate (IPCC) activation In situ alcoholysis of the unstable mixed anhydride intermediate was catalyzed by 4-(dimethylamino)pyridine (DMAP) Competing isopropenyl ester formation was negligible when using methylene chloride as the solvent A variety of esters from primary and secondary alcohols were obtained with good yields (60 to 96 %), and even the more hindered tertiobutyl alcohol gave acceptable yields under more drastic conditions The improvement in depsipeptide synthetic methodology is illustrated by preparation of the antibiotic valinomycin, using IPCC for ester bond formation, and BOP reagent for peptidic coupling and the last-step cyclization

Introduction

Esters of amino acids are often used as the starting point for peptide elongation [2] Ester linkage is also found in numerous compounds of biological importance [3] Despite the wealth of available methods for amino ester preparation,[4] only few simple processes allow esterification by alcoholysis of the activated carboxylic function, under mild conditions [5] Until to now, the synthesis of depsipeptides from N-protected amino acids has best been achieved using the carboxylate alkylation[4e] or alternatively DCC[5b] and its water-soluble analogue EDCI[5c] with DMAP as the catalyst However, the method has drawbacks, such as the formation of by products, difficulties in purification, and the assumed racemization [5a,c,6] A recent improvement using DMAP TFA as an additive in refluxing chloroform permits macrolactonization of the cyclodepsipeptide (+) Jasplakinolide [7]

As part of an ongoing program devoted to developing general methods for the construction of biologically important depsipeptides,[8] we have investigated the esterification of N-protected amino acids using isopropenyl chlorocarbonate (IPCC) activation[9,10] and our preliminary results concerning DMAP-catalyzed alcoholysis of mixed carboxylic-carbonic anhydride has previously been reported [10] This activated α-amino acid was generated in situ from equimolar amounts of IPCC and triethylamine (Scheme 1, eq 1)

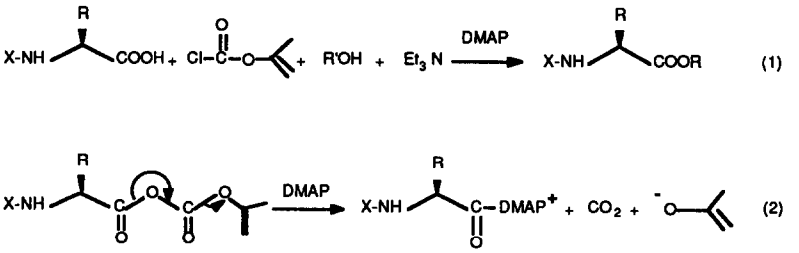
The present paper details the preparation of amino esters and the synthesis of depsipeptides This reaction is proposed for use in a new method for preparing the antibiotic valinomycin **1** (Figure)

Results and Discussion

Isopropenyl chlorocarbonate has been used as a carboxylic activator in classical peptide synthesis in solution [9] As with the usual alkyl chlorocarbonates, the formation of a transient mixed anhydride was

postulated [11] Mixed carboxylic-carbonic anhydrides are known to generate the corresponding alcohol as a by-product In a recent study, Kim *et al* took advantage of this side reaction to prepare esters from alkyl chlorocarbonates, using DMAP as the catalyst [5d] However, the procedure was limited by the accessibility of the required chlorocarbonates In contrast, mixed anhydride prepared from IPCC releases the enolate of acetone (Scheme 1, eq 2)

Scheme 1. IPCC Promoted Esterification of Aminoacids



To account for this reaction, formation of the corresponding isopropenyl ester was investigated by treating tert-butyloxycarbonyl-phenylalanine (BocPhe) with 1 equiv of IPCC, 1 equiv of triethylamine, and 0 2 equiv of DMAP in different solvents at -5 °C The results summarized in Table I show that isopropenyl-tert-butyloxycarbonyl-phenylalaninate 2 was not formed in methylene chloride and that medium yields were obtained in ether-type solvents (Scheme 2) Therefore, the use of IPCC in methylene chloride remained very promising for nucleophilic coupling of N-protected amino acids with hydroxyl compounds, excluding isopropenyl ester formation

Scheme 2. Formation of Isopropenylester

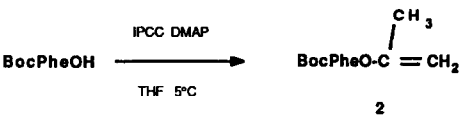


Table I. Solvent Effect on the Formation of Boc-Phe-O-isopropenyl 2

Solvent	Yield %
CH_2Cl_2	0
THF	59
Dioxane	44
DME	45

It was then necessary to compare IPCC with other chlorocarbonates in the alcoholysis of the mixed anhydride intermediate by a hydroxyl derivative Its superiority was demonstrated in the following experiment BocPhe was activated either with IPCC or with methyl chlorocarbonate in the presence of deuterated methanol The amounts of deuterated methyl ester formed were clearly measured by ^1H NMR integration The results summarized in Table II show that activation with IPCC led exclusively to the corresponding deuterated ester (entry 1), whereas activation with methyl chlorocarbonate gave a mixture of deuterated and non-deuterated methyl esters (entry 4) even when using a

large excess of deuterated methanol (entry 5)

Table II. Comparison of IPCC and Methyl Chlorocarbonate (MCC)[a]

entry	amino acid	alcohol	chlorocarbonate	ratio of ester (%) non deuterated	deuterated
1	Boc-Phe-OH	DO-CD_3	IPCC	0	100
2		HO-CH_3	IPCC	100	0
3		none	MCC	100	0
4		DO-CD_3	MCC	30	70
5		DO-CD_3 [b]	MCC	7	93

(a) -All reactions were carried out with equimolar amounts of amino acid, chlorocarbonate, alcohol, and triethyl amine using 0 2 equiv of DMAP (b) - 5 equiv of alcohol was used

N-protected α-amino esters have been prepared from a variety of amino acids using primary alcohols (Table III) and secondary alcohols (Table IV) We found that even acid-sensitive alcohols such as S-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol can be used efficiently (Table III, entries 9, 13, 14) This method also has the advantage of allowing esterification of amino acids with tertiary alcohols N-protected α-amino tert-butyl esters are currently prepared either by reacting the benzyloxycarbonyl derivative with isobutene and concentrated sulfuric acid,[12] or by coupling the acid with tert-butyl alcohol in the presence of a carbodiimide and 4-(dimethylamino)-pyridine [5c] Alternatively N,N-dimethylformamide di-tert-butyl acetal has sometimes

Table IV. Preparation of Secondary Esters of N-Protected Amino Acids

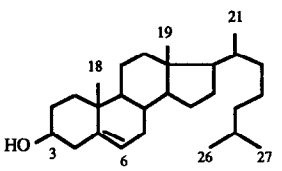
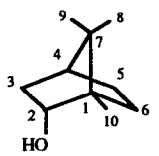
amino acid	alcohol	yield, %	mp, °C	$[\alpha]_D^{20}$ (1, MeOH)
Boc-Val-OH		87	56-57	-75
Boc-Val-OH		82	133-134	-40

Table III Preparation of Primary Esters of N-Protected α - Amino Acids [a]

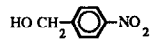
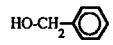
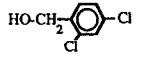
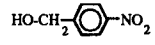
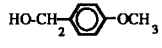
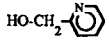
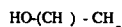
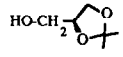
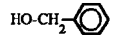
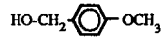
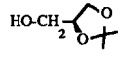
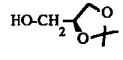
entry	amino acid	alcohol	yield, %	mp, °C (lit.)	$[\alpha]_D^{20}$ (lit) [b]	360 MHz ^{1}H N.M.R (in $CDCl_3$, δ ppm)
1	Z-Ala-OH		78	99 (98-99) [17]	-16 3(-16 8 [1,MeOH]) [17]	1 8 (3H, d, CH_3), 4 8 (1H, m, H_α), 5 2 (2H, s, CH_2Z), 5 5 (2H, s, CH_2Bzl-NO_2), 5 5 (1H, d, NH), 7 6 (5H, m, Z), 7 9 (2H, d) and 8 4 (2H, d, Bzl-NO_2)
2	Boc Val-OH		90	oil	40 (-33 3 [2, MeOH]) [5c]	0 82 (3H, d) and 0 92 (3H, d, CH_3 Val), 1 4 (9H, s, Boc), 2 1 (1H, m, H_β), 4 5 (1H, m, H_α), 5 1 (1H, d, NH), 5 3 (2H, q, CH_2Bzl), 7 4 (5H, m, Bzl)
3			85	59-60	-25 1	0 8 (3H, d) and 0 93 (3H, d, CH_3 Val), 1 42 (9H, s, Boc) 2 2 (1H, m, H_β), 4 25 (1H, m, H_α), 5 0 (1H, d, NH), 5 2 (2H, q, CH_2Bzl-Cl_2), 7 43 (3H, m, Bzl-Cl_2)
4			70	65-66	-24 5	0 8 (3H, d) and 0 9 (3H, d, CH_3 Val), 1 4 (9H, s, Boc), 2.2 (1H, m, H_β), 4 25 (1H, m, H_α), 4 9 (1H, d, NH), 5 25 (2H, q, CH_2Bzl-NO_2), 7 5 (2H, d) and 8 2 (2H, d, Bzl-NO_2)
5			91	oil	-24	0 8 (3H, d) and 0 92 (3H, d, CH_3 Val), 1 42 (9H, s, Boc), 2 1 (1H, m, H_β), 3 78 (3H, s, OCH_3), 4.25 (1H, m, H_α), 4 95 (1H, d, NH), 5 05 (2H, q, CH_2Bzl-OCH_3), 6.85 (2H, d) and 7 25 (2H, d, Bzl-OCH_3)
6			72	oil	-30 7	0 8 (3H, d) and 0 9 (3H, d, CH_3 Val), 1 4 (9H, s, Boc) 2.2 (1H, m, H_β), 4 25 (1H, m, H_α), 5 05 (1H, d, NH), 5 2 (2H, q, CH_2Pyr), 7 25 (1H, d), 7 6 (1H, d) and 8 25 (2H, d, Pyr)
7			68	98-99	-27 8	0 8 (3H, d) and 0 9 (3H, d, CH_3 Val), 1 4 (9H, s, Boc), 2 1 (1H, m, H_β), 4 25 (1H, m, H_α), 5 (1H, d, NH), 6 2 (2H, d, CH_2Ant), 7.5 (4H, m), 8 (2H, d) and 8 25 (2H, d, Ant), 8.5 (1H, s, Ant)
8			96	oil	-13 9	0 82 (3H, d) and 0 9 (3H, d, CH_3 Val), 0 83 (3H, t, CH_3 cetyl), 1 25 (28H, m, CH_2 cetyl), 1.4 (9H, s, Boc), 1 61 (2H, m, CH_2 cetyl), 2 1 (1H, m, H_β), 4 1 (2H, m, OCH_2 cetyl), 4 15 (1H, m, H_α) 5 (1H, d, NH)
9			75	42	-12	0 81 (3H, d) and 0 9 (3H, d, CH_3 Val), 1 28 (3H, s) and 1.37 (3H, s, CH_3 glyceryl), 1 4 (9H, s, Boc), 2 05 (1H, m, H_β), 3 7 (1H, q) and 3 98 (1H, q, CH_2 glyceryl), 4 07 (2H, m, OCH_2 glyceryl), 4 15 (1H, m, H_α), 4 21 (1H, m H glyceryl), 4 96 (1H, d, NH)

Table III Preparation of Primary Esters of N-Protected α - Amino Acids (continued)

10	Boc-Phe-OH	HO-CH_2- 	92	64-65 (64-65) [5c]	12 (-12.8 [2, MeOH]) [5c]	1 41 (9H, s, Boc), 3 1 (2H, d, CH_2 Phe), 4 4 (1H, m H_α), 5 2 (2H, s, CH_2 Bzl), 5 3 (1H, d, NH) 7 2 (10H m, Phe and Bzl)
11		HO-CH_2- 	82	61-62	-10	1 38 (9H, s, Boc), 3 05 (2H, m, CH_2 Phe), 3 8 (3H, s, OCH_3), 4.58 (1H, m, H_α), 4 95 (1H, d, NH) 5 05 (2H s, CH_2 Bzl-OCH_3), 6 85 (2H, d) and 7 01 (2H, d, Bzl-OCH_3), 7 2 (5H, m, Phe)
12		HO-$(CH_2)_{14}$-CH_3	96	38-39	-2 6	0 85 (3H, t, CH_3 cetyl), 1 25 (26H, m, CH_2 cetyl), 1 4 (9H, s, Boc), 1 61 (2H,m, CH_2 cetyl), 3 1 (2H, m, CH_2 Phe), 4 1 (2H, m, OCH_2 cetyl), 4 6 (1H, m, H_α), 4 9 (1H, d, NH), 7 25 (5H, m, Phe)
13		HO-CH_2- 	80	85-86	-2	1 35 (3H, s) and 1 39 (3H, s, CH_3 glyceryl), 1 4 (9H, s, Boc), 3 07 (2H, m, CH_2 Phe), 3 6 (1H, q) and 3 95 (1H, q, CH_2 glyceryl), 4 15 (2H, m, OCH_2 glyceryl), 4.25 (1H, m, H glyceryl), 4 6 (1H, m, H_α), 4 95 (1H, d, NH), 7 2 (5H, m, Phe)
14	Fmoc-Trp-OH	HO-CH_2- 	65	61-62	-12 [1, DMF]	1 32 (3H, s) and 1 41 (3H, s, CH_3 glyceryl), 3 3 (2H, m, CH_2 Trp), 3 45 (1H, t) and 3 9 (1H, t, CH_2 glyceryl), 4 1 (t, 1H, H Fmoc), 4 25 (2H, m, OCH_2 glyceryl), 4 4 (2H, m, CH_2 Fmoc), 4 45 (1H, m, H glyceryl), 4 7 (1H, m, H_α), 5 3 (1H, d, NH), 6 9 - 8 1 (14H, m, Trp and Fmoc)
15	Boc-Asp(OBzl)-OH	$HOCH_3$	74	61-62 (67-68) [5d]	-7 (-7 1 [1, acetone]) [5d]	1 42 (9H, s, Boc), 2 9 (2H, m, β CH_2), 3 7 (3H, s, OCH_3), 4 58 (1H, m, H_α), 5 1 (2H, q, CH_2 Bzl), 5 45 (1H, d, NH), 7 32 (5H, m, Bzl)
16	Z-Gaba-OH	HO-$(CH_2)_{14}$-CH_3	87	50		0 86 (3H t, CH_3 cetyl), 1 25 (26H, m, CH_2 cetyl), 1 58 (2H, m, CH_2 cetyl), 1 82 (2H, β CH_2), 2 9 (2H, t, α CH_2), 3 21 (2H, q, γ CH_2), 4 05 (2H, m, OCH_2 cetyl), 4 87 (1H, m, NH), 5 07 (2H, s, CH_2 Z), 7 3 (5H, m, Z)

(a)- All new compounds gave satisfactory elemental analytical data and their molecular masses were confirmed by FAB mass spectra.

(b)- All specific rotations were measured in methanol at 20°C at a concentration of 1 g/100ml if not mentioned.

Table V Preparation of *tert* -Butyl Esters of N-Protected α -Amino Acids

entry	ester	method [a]	yield %	mp, °C (lit)	$[\alpha]_D^{20}$ (lit) [b]
1	Boc-Ala-OtBu	A	50	oil	-38 1 (-32.1) [18]
2	Boc-Ala-OtBu	B	60	oil	-36
3	Z-Ala-OtBu	A	60	oil	-19 5 (-15 8 [2 1, MeOH]) [5c]
4	Boc-Asp(OBzl)-OtBu	A	45	50	-6 (-7 4 [2, MeOH]) [5c]
5	Z-Cys(SBzl)-OtBu	B	75	oil	-30
6	Z-Lys(ε Boc)-OtBu [19]	B	70	oil	-90
7	Z-Met-OtBu	B	68	oil	-22.9 (-27 [5 7, EtOH]) [12]
8	Boc-Met-OtBu	B	50	35	-33 5
9	Z-Phe-Otbu	A	70	81-82 (81-82)	-9 9 (-6 [2, EtOH]) [20]
10	Z-Phe-OtBu	B	88	82	-9 9
11	Z-Pro-OtBu	A	90	43 (44-45)	-51 (-52.5 [2, EtOH], [12]
12	Z-Pro-OtBu	B	63	44	-52
13	Z-Val-OtBu [12]	A	45	oil	-6
14	Z-Trp-OtBu	B	60	70-71	-ɔ 2
15	Z-Leu-OtBu [12]	A	84	oil	-15 3

(a)- See experimental section (b)- All specific rotations were measured in methanol at a concentration of 1g/100ml

Table VI Synthesis of Depsipeptides using IPCC-DMAP

R-COOH	HO-R'	yield%	mp,°C	$[\alpha]_D^{20}$ [a]
Boc-Phe-OH	Z-Thr-OMe	95	oil	+8
Boc-Val-OH	Z-Thr-OMe	96	oil	-7
Boc-Phe-OH	Z-Ser-OMe	80	oil	-12
Boc-Tyr(Me)-OH	Z-Thr-CAM [b]	98	53-55	+5
Boc-MeTyr(Me)-OH	Z-Thr-CAM [b]	97	48-49	-20
Boc-MeTyr(Me)-OH	Z-Thr-OAllyl	98	oil	-23
Boc-D-Ala-OH	L-Hyv-OBzl	92	oil	+8
Boc-Ala-OH	L-Hyv-OBzl	95	oil	-67
Boc-D-Val-OH	L-Lac-OBzl	98	oil	-9
Boc-Val-OH	D-Hyv-OBzl	90	oil	-8

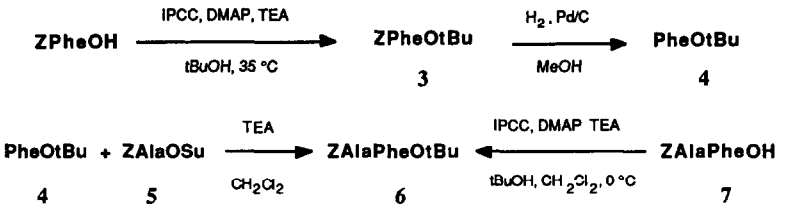
(a) - All specific rotations were measured in methanol at a concentration of 1g/100ml.

(b) - Carboxamido ester (CAM) was synthesized according to Martinez [21]

been used [13] In the preparation of tert-butyl esters using IPCC activation, two slightly different procedures were followed (i) an equimolar mixture of amino acids, IPCC, triethylamine, and tert-butyl alcohol in methylene chloride were stirred with 0 2 equiv of DMAP for 30 min at 0°C (method A), or (ii) tert-butyl alcohol was used as the solvent and the reaction was done for the same time at 30°C (method B) Under the latter conditions, increasing yields were generally observed (Table V)

Epimerization must be absent to ensure the usefulness of the method, and initial proof of this was provided by the observation that the optical rotations of the isolated esters were comparable to those reported in the literature However, these specific rotations are usually low and the small variations given in the different reports are not significant Thus, unambiguous proof of the lack of epimerization was obtained by ^{1}H NMR, using the primary S-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol (entries 9, 13, 14, Table III) and secondary

Scheme 3. Synthesis of ZAlaPheOtBu



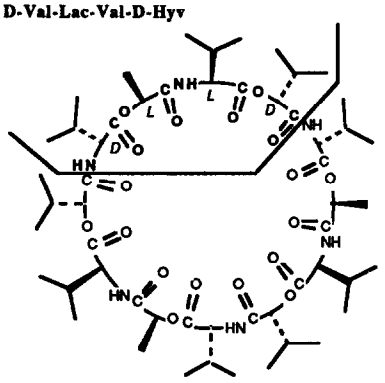
cholesterol or R-(+)-borneol (Table IV) The extent of epimerization for tert-butyl ester formation was measured by diastereomeric ratios In this study, both tert-butyl esters of the N-benzyloxycarbonyl-L-phenylalanine (ZPheOtBu **3**) and the corresponding D-isomer were prepared using the more drastic method B, after hydrogenolysis, the resulting free amine **4** was coupled with the hydroxy succinimid-active ester of N-benzyloxycarbonyl-L-alanine (ZAlaOSu **5**) In the ^{1}H-NMR spectra, signals related to the tert-butyl esters of phenylalanine or the β-methyl of alanine were clearly separate from each of the two diastereomers **6**, and no mixed spectra were obtained for either dipeptide, thus indicating an epimerization lower than 2 % In contrast, tert-butyl esterification of ZAlaPhe **7** showed 25% epimerization of the stereogenic C-α of the Phe residue This last expected result shows the limitation of the method for N-acyl amino acid esterification

Synthesis of depsipeptides is given here as a general application of this ester bond formation Following normal IPCC activation, all depsipeptides were produced with excellent yields (Table VI) The method was often favorable compared to DCC or the recently published COMODD activation [14] Taking advantage of the presence of two chiral centers, the diastereomeric purity of the isolated compounds was ascertained by ^{1}H NMR

Total synthesis of the antibiotic valinomycin **1**[15] was undertaken to show the reliability of the method

Figure. Valinomycin

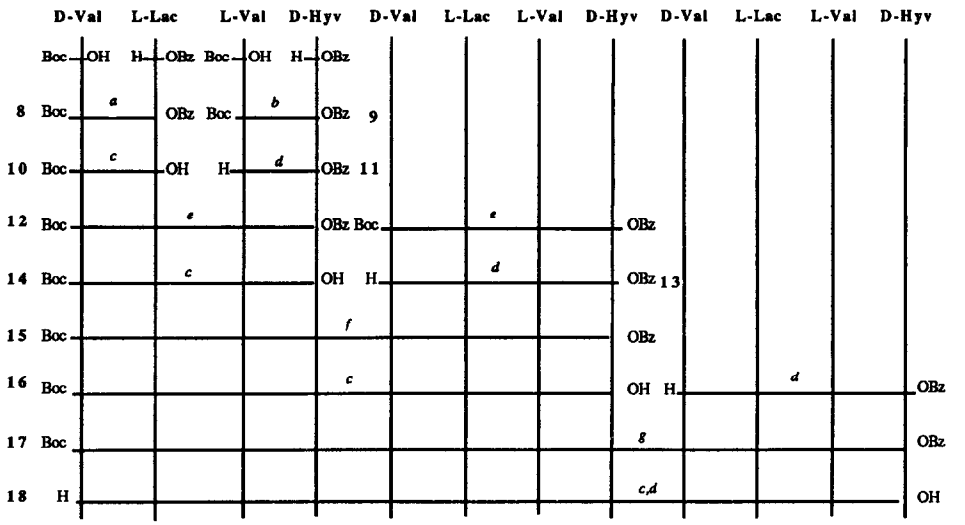
D-Val-Lac-Val-D-Hyv



1

In the Figure it can be seen that this dodeca-cyclodepsipeptide is a trimeric molecule Based on previously published strategies,[15c] it appeared advisable to choose the tetradepsipeptide D-Val L-Lac L-Val D-Hyv subunit for the segment-coupling formation of the linear precursor Depsipeptides D-Val-Lac and Val-D-Hyv were needed for the convergent synthesis of this tetradepsipeptide, as depicted in Scheme 4 Thus, following IPCC activation, Boc-D-Val-Lac-OBzl **8** and Boc-Val-D-Hyv-OBzl **9** were prepared with 98% and 90% yields, respectively After the usual deprotections, Boc-D-Val-Lac **10** was coupled to TFA.Val-D-Hyv-OBzl **11** by the BOP procedure,[16] giving the tetradepsipeptide Boc-D-Val-Lac-Val-D-Hyv-OBzl **12** with an 80% yield With one batch of this tetradepsipeptide **12**, Boc protection was removed by TFA, leading to TFA D-Val-Lac-Val-D-Hyv-OBzl **13**, and with another batch, the carboxylic benzyl protection was removed by hydrogenation, leading to Boc-D-Val-Lac-Val-D-Hyv **14** These two products were used for repetitive segment coupling

Scheme 4. The Synthesis of Valinomycine



[a] IPCC/DMAP, 98% [b] IPCC/DMAP 90% [c] H_2/Pd-C [d] TFA. [e] BOP/TEA, 80% [f] BOP/TEA, 80%
[g] BOP/TEA, 90%

elongation The octa-depsipeptide intermediate **15** was isolated with an 80% yield and the linear dodeca-depsipeptide **16**, the precursor of valinomycin **1**, was obtained with a 90% yield using the BOP procedure Finally, after deprotection of both termini, BOP-promoted cyclization led to crystallized valinomycin with a moderate yield (30%) using no further improvements This synthetic compound exhibited data comparable to the valinomycin described in the literature, and was identical to a commercially available sample

Experimental Section

Melting points were determined using a Buchi melting-point apparatus NMR data were obtained at 360 MHz on a Bruker WM-360 instrument, chemical shifts (ppm) were reported relative to internal tetramethylsilane Specific optical rotations were measured on a Schmidt and Haensch Polartronic D apparatus and are at ±1° Elemental analyses were obtained from the Service Central d'Analyse du CNRS FAB mass measurements were supplied by Pr Aubagnac, USTL Montpellier Analytic TLC were performed on silica gel F254 aluminium sheets (0 2 mm thick, Merck) Column chromatographies were performed using silica gel (70-200 m, Amicon) BOP reagent was a gift from Sempa-Chimie IPCC was obtained, as a gift, from SNPE (France) Amino acid derivatives were purchased from Bachem or Novabiochem Valinomycin was purchased from Aldrich

Isopropenyl N-Boc-phenylalaninate IPCC (0.25 ml, 2 2 mmol), was added at 0°C to a solution of Boc Phe (530 mg, 2 mmol), TEA (0 27 ml, 2 mmol) and DMAP (25 mg, 0 2 mmol) in THF (10 ml) After 1 h of stirring, ether (20 ml) was added and the solution was washed with water, 5% sodium hydrogenocarbonate, and saturated brine The organic phase was dried over anhydrous sodium sulfate and evaporated to dryness Silicagel chromatography of the crude residue (hexane - ethyl acetate, 85 15) afforded 360 mg of isopropenyl ester yield 59%, m p 55°C (ether - hexane), Rf 0 5 (hexane - ethyl acetate 80 20), $[\alpha]^{20}D$ -8° (c 1, MeOH), ^1H NMR ($CDCl_3$) δ ppm 1 4 (9H, s, Boc), 3 1 (2H, m, CH_2 Phe), 4 6 (2H, m, Hα Phe and H isopropenyl), 4 7 (1H, s, H isopropenyl), 4 95 (1H, m, NH), 7 2 (5H, m, Phe) Calc for C17H23NO4 C 66 86, H 7 59, N 4 59 % Found C 66 61, H 7 68, N 4 51 MS 306 (MH+), 250 (28%), 206 (20%),120 (58%), 57 (100%)

General procedure of esterification with primary and secondary alcohols TEA (0 75 ml, 5 5 mmol) and DMAP (122 mg, 1 mmol) were added to a solution of the N-protected amino acid (5 mmol) and the alcohol (5.5 mmol) in methylene chloride (15 ml) The mixture was cooled to 0°C and IPCC (0 65 ml, 5.5 mmol) was added dropwise with stirring for 10 min After an additional 20 min, ethyl acetate (50 ml) was added, then washed with potassium hydrogenosulfate 5 % (2 x 10 ml), sodium hydrogenocarbonate 5 % (2 x 10 ml), and saturated brine (10 ml) The organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure The product was purified either by crystallization or by column chromatography using mixtures of ethyl acetate and hexane as the eluent

Boc-Val-O-cholesteryl 87% yield, mp 56-57 °C, $[\alpha]^{20}D$ -75° (c 1, MeOH), ^1H NMR ($CDCl_3$) δ ppm 0 67 (3H, s, CH_3-18), 0 82 (3H, d) and 0 92 (3H, CH_3-Val), 0 87 (6H, d, CH_3 - 26, 27), 0 92 (3H, s, CH_3 - 21), 1 4 (9H, s, Boc), 2 2 (1H, m, Hβ), 4 15 (1H, m, H - 3) 4 65 (1H, m, Hα), 5 05 (1H, d, NH), 5 37 (1H, d, H-6) Other signals were not attributed Calc for C37H63NO4 C 75 85, H 10 84, N 2 39 % Found C 75 98, H 10 96, N 2 22

Boc-Val-O-bornyl 82% yield, mp 133-134 °C, $[\alpha]^{20}D$ -40° (c 1, MeOH), ^1H NMR ($CDCl_3$) δ ppm 0 79 (3H, s, CH_3-10), 0 80 (3H, d) and 0 91 (3H, CH_3-Val), 0 86 (3H, d) and 0 88 (3H, d, CH_3 - 8, 9), 0 97 (1H, m, H - 3a), 1 27 (1H, m, H- 5a), 1 3 (1H, m, H - 6a), 1 4 (9H, s, Boc), 1 65 (1H, t, H - 4), 1 74 (1H, m, H - 5e), 1 9 (1H, m, H - 3e), 2 14 (1H, m, Hβ), 2 35 (1H, m, H - 2), 4 22 (1H, m, Hα), 4 86 (1H, m, H - 6), 5 0 (1H, d, NH) Calc for C20H35NO4 C 67 95, H 9 98, N 3 96 % Found C 67 83, H 10 16, N 4 04

General procedure of esterification with tert-butyl alcohol

Method A This method is identical to the foregoing procedure apart from the amount of tertiobutanol (3 molar equivalent)

Method B TEA (0 75 ml, 5 5 mmol) and DMAP (122 mg, 1 mmol) and then IPCC (0 65 ml, 5 5 mmol) were added to a heated (35 °C) solution of the N-protected amino acid (5 mmol) in tertiobutanol After 30 min, the solution was concentrated under reduced pressure The residue was solubilized in ethyl acetate (50 ml) and worked up as described above

Preparation of Z-Ala-Phe-OtBu Pd/C catalytic hydrogenation of Z-Phe-OtBu, prepared according to method B (335 mg, 1 mmol), in MeOH gave the free amine after 1 h at room temperature and atmospheric pressure The reaction mixture was then filtered on celite and the solution evaporated under reduced pressure The solution of this free amine and Z-Ala-OSu[22] (320 mg, 1 1 mmol) in dichloromethane (10 ml) was cooled to 0°C DIEA (0 18 ml, 1 mmol) was added dropwise to the solution with stirring

After 1 h, the solution was washed with 5% potassium hydrogenosulfate (2 x 10 ml), 5% sodium hydrogenocarbonate (2 x 10 ml), and saturated brine (10 ml) The organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure The dipeptide was isolated as a colorless oil (90%), which was homogeneous according to TLC analysis (ethyl acetate / hexane 50.50 , Rf 0.53), ^{1}H NMR (DMSO, D_6) δ 1 19 (3H, d, CH_3 Ala), 1 3 (9H, s, OtBu), 2 95 (2H, m, CH_2 Phe), 4 12 (1H, m, H-α Phe), 4 37 (1H, m, H-α Ala), 5 0 (2H, s, CH_2 Z), 7 1 - 7 4 (11H, m, C_6H_5 Z, C_6H_5 Phe and NH Phe), 8 09 (1H, d, NH Ala)

Z-Ala-D-Phe-OtBu was prepared according to the same procedure starting from Z-D-Phe-OtBu ^{1}H NMR (DMSO, D_6) δ 1 04 (3H, d, CH_3 Ala), 1 37 (9H, s, OtBu), 2 91 (1H, m) and 3 04 (1H, m, CH_2 Phe), 4 08 (1H, m, H-α Phe), 4 4 (1H, m, H-α Ala), 5 0 (2H, s, CH_2 Z), 7 1 - 7 4 (11H, m, C_6H_5 Z, C_6H_5 Phe and NH Phe), 8 2 (1H, d, NH Ala)

Z-Ala-Phe-OH tert-butylation Z-L-Ala-Phe-OH (369 mg, 1 mmol) was esterified according to method A The usual washings gave the tert-butyl ester (255 mg, 60% yield) ^{1}H NMR spectra showed two sets of signals corresponding to Z-Ala-Phe-OtBu (80%) and Z-Ala-D-Phe-OtBu (20%)

Depsipeptide preparation: IPCC (1 1 eq) was added to a solution of N-protected amino acid, C-protected hydroxy acid (1 eq), TEA (1 4 eq) and DMAP (0 4 eq) in dichloromethane, at 0°C After 1 h of stirring, the solution was washed with water, 5% sodium hydrogenocarbonate, and saturated brine The organic phase was dried over anhydrous sodium sulfate and evaporated to dryness The depsipeptide was purified by silicagel column chromatography

Z-Thr-(Boc-Phe)-OMe was prepared from Z-Thr-OMe and Boc-Phe as a colorless oil (95% yield), which was homogeneous according to TLC analysis (ethyl acetate / hexane 50 50, Rf 0 83), $[\alpha]^{20}_D$ +8° (c 1, MeOH), ^{1}H NMR (DMSO, D_6) δ 1 13 (3H, d, J = 6 1 Hz, CH_3 Thr), 1 29 (9H, s, Boc), 2 75 (1H, dd, J_1 = 9 8 Hz, J_2 = 13 4 Hz), and 2 95 (1H, dd, J_1 = 5.2 Hz, J_2 = 13 4 Hz, H-β Phe), 3 65 (3H, s, OCH_3), 4 15 (1H, m, H–α Phe), 4 45 (1H, dd, J_1 = 2 4 Hz, J_2 = 9 2 Hz, H–α Thr), 5 11 (2H, s, CH_2 Z), 5 25 (1H, m, H-β Thr), 7 3 (11H, m, C_6H_5 Z, C_6H_5 Phe and NH Phe), 7 65 (1H, d, J = 9 2 Hz, NH Thr) MS 515 (MH^+), 415 (25%), 120 (28%), 91 (100%), 57 (55%)

Z-Thr-(Boc-Val)-OMe was prepared from Z-Thr-OMe and Boc-Val as a colorless oil (96% yield), which was homogeneous according to TLC analysis (ethyl acetate / hexane 50 50, Rf 0 76), $[\alpha]^{20}_D$ -7° (c 1, MeOH), ^{1}H NMR (DMSO, D_6) δ 0 79 (3H, d, J = 6 7 Hz) and 0 81 (3H, d, J = 6 7 Hz, CH_3 Val), 1 15 (3H, d, J = 6 1 Hz, CH_3 Thr), 1 36 (9H, s, Boc), 1 85 (1H, m, H-β Val), 3 62 (3H, s, OCH_3), 3 78 (1H, m, H-α Phe), 4 45 (1H, dd, J_1 = 3 1 Hz, J_2 = 9 8 Hz, H-α Thr), 5 2 (1H, m, H–β Thr), 7 11 (1H, d, J = 9 8 Hz NH Val), 7 26-7 41 (5H, m, C_6H_5 Z), 7 69 (1H, d, J = 9 2 Hz, NH Thr) MS 467 (MH+), 367 (20%), 91 (100%), 72 (43%), 57 (44%)

Z-Ser-(Boc-Phe)-OMe was prepared from Z-Ser-OMe and Boc-Phe as a colorless oil (85% yield), which was homogeneous according to TLC analysis (ethyl acetate / hexane, 50 50, Rf 0 84), $[\alpha]^{20}_D$ -12° (c 1, MeOH), ^{1}H NMR (DMSO, D_6) δ 1 3 (9H, s, Boc), 2 9 (2H, m, H-β Phe), 3 67 (3H, s, OCH_3), 4 19 (1H, m, H–α Phe), 4 3 (2H, m, CH_2 Ser), 4 45 (1H, m, H-α Ser), 5 06 (2H, s, CH_2 Z), 7 15 (1H, m, NH Phe), 7 4 (10H, m, C_6H_5 Z and C_6H_5 Phe), 7 82 (1H, d, J = 8 3 Hz, NH Ser) MS 501 (MH+), 401 (24%), 120 (21%), 91 (100%), 57 (56%)

Z-Thr-[Boc-Tyr(Me)]-CAM was prepared from Z-Thr-CAM and Boc-Tyr(Me) as a white powder (98% yield), which was homogeneous according to TLC analysis (ethyl acetate / hexane / acetic acid 50 50 1, Rf 0 35), $[\alpha]^{20}_D$ +5° (c 1, MeOH), ^{1}H NMR (DMSO, D_6) δ 1 16 (3H, d, J = 6 1 Hz, CH_3 Thr), 1 30 (9H, s, Boc), 2 69 (1H, dd, J_1 = 9 8 Hz, J_2 = 13 7 Hz), and 2 9 (1H, dd, J_1 = 4 9 Hz, J_2 = 13 7 Hz, H-β Tyr), 3 71 (3H, s, OCH_3), 4 1 (1H, m, H-α Tyr), 4 36 and 4.55 (2H, d, J = 15 1 Hz, CH_2 CAM), 4 61 (1H, dd, J_1 = 2 8 Hz, J_2 = 9 3 Hz, H-α Thr), 5 12 (2H, s, CH_2 Z), 5 32 (1H, m, H-β Thr), 6 83 and 7 11 (2H, d, J = 8 4 Hz, C_6H_4 Tyr), 7 09 (1H, d, J = 7 Hz, NH Tyr), 7 31-7 44 (5H, m, C_6H_5 Z), 7 75 (1H, d, J = 9 4 Hz, NH Thr) Calc for $C_{29}H_{37}N_3O_{10}$ C 59 28, H 6 35, N 7 15 % Found C 59 12, H 6 58, N 6 98 MS 588 (MH+), 488 (15%), 121 (35%), 91 (100%), 57 (50%)

Z-Thr-[Boc-MeTyr(Me)]-CAM was prepared from Z-Thr-CAM and Boc-MeTyr(Me) as a white powder (98% yield), which was homogeneous according to TLC analysis (ethyl acetate / hexane / acetic acid 50 50 1, Rf 0 52), $[\alpha]^{20}_D$ -20° (c 1, MeOH), ^{1}H NMR (DMSO, D_6) δ 1 20 (3H, d, J = 6 1 Hz, CH_3 Thr), 1 30 (9H, s, Boc), 2 56 (3H, s, N-CH_3 Tyr), 2 82-2 94 (2H, m, H-β Tyr), 3 70 (3H, s, OCH_3), 4 40 and 4 51 (2H, d, J = 15 1 Hz, CH_2 CAM), 4 50-4 83 (2H, m, H-α Thr and H-β Tyr), 5 08 (2H, s, CH_2 Z), 5 25 (1H, m, H-β Thr), 6 82 and 7 09 (2H, d, J = 8 5 Hz, C_6H_4 Tyr), 7 24-7.55 (5H, m, C_6H_5 Z), 7 80 (1H, d, J = 9 2 Hz, NH Thr), 7 89 (NH Thr, minor rotamer) Calc for $C_{30}H_{39}N_3O_{10}$ C 59 89, H 6.53, N 6 98 % Found C 59 75, H 6 50, N 6 92

Boc-D-Ala-Hyv-OBzl was prepared from Hyv-OBzl and Boc-D-Ala as a colorless oil (92% yield), which was homogeneous according to TLC analysis (ethyl acetate / hexane 20 80; Rf 0 42), $[\alpha]^{20}_D$ +8° (c 1, MeOH), ^{1}H NMR (DMSO, D_6) δ 0 88 (3H, d, J = 6 7 Hz) and 0 92 (3H, d, J = 6 7 Hz, CH_3 Hyv), 1 25 (3H, d, J = 7 3 Hz, CH_3 Ala), 1 38 (9H, s, Boc), 2 15 (1H, m, H-β Hyv), 4 1 (1H, m, H-α Ala), 4 81 (1H, m, H-α Hyv), 5 14 and 5 18 (1H, d, J = 12 3 Hz, CH_2 Bzl), 7 27-7 42 (6H, m, C_6H_5 Bzl and NH Ala) MS 380 (MH+), 280 (42%), 91 (100%), 57 (53%)

Boc-Ala-Hyv-OBzl was prepared from Hyv-OBzl and Boc-Ala as a colorless oil (95% yield), which was homogeneous

according to TLC analysis (ethyl acetate / hexane 20 80; Rf 0 45), $[\alpha]^{20}_D$ -67° (c 1 16, MeOH), ^{1}H NMR (DMSO, D_6) δ 0.88 (3H, d, J = 6 7 Hz) and 0 94 (3H, d, J = 6 7 Hz, CH_3 Hyv), 1 25 (3H, d, J = 7 Hz, CH_3 Ala), 1 38 (9H, s, Boc), 2 15 (1H, m, H-β Hyv), 4 07 (1H, m, H-α Ala), 4 68 (1H, m, H-α Hyv), 5 12 and 5 19 (1H, d, J = 12 3 Hz, CH_2 Bzl), 7.25-7 40 (6H, m, C_6H_5 Bzl and NH Ala)

Preparation of valinomycin 1

Boc-D-Val-Lac-OBzl. (8)[15c] was prepared from Boc-D-Val and Lac-OBzl as a colorless oil (98% yield), which was homogeneous according to TLC analysis (ethyl acetate / hexane 5 95, Rf 0.5), $[\alpha]^{20}_D$ -9.5° (c 2, benzene), ^{1}H NMR ($CDCl_3$) δ 0 88 (3H, d) and 0 96 (3H, d, CH_3 Val), 1 42 (9H, s, Boc), 1 5 (3H, d, CH_3 Lac), 2 2 (1H, m, H-β Val), 4 3 (1H, m, H-α Val), 4 95 (1H, m, NH Val), 5 15 (3H, m, H-α Lac and CH_2 Bzl), 7 32 (5H, m, C_6H_5 Bzl)

Boc-D-Val-Lac-OH (10) [15c] was prepared by Pd/C catalytic hydrogenation of Boc-D-Val-Lac-OBzl (760 mg, 2 mmol) in MeOH for 4 h at room temperature and under atmospheric pressure The reaction mixture was then filtered on celite and the solution evaporated under reduced pressure to give 550 mg of a compound (95% yield) identical to previously described Boc-D-Val-Lac-OH

Boc-Val-D-Hyv-OBzl (9)[15c] was prepared following IPCC-promoted depsipeptide formation from D-Hyv-OBzl and Boc-Val, as a colorless oil (90% yield), which was homogeneous according to TLC analysis (ethyl acetate / hexane 20 80, Rf 0 56), $[\alpha]^{20}_D$ -8° (c 1, dioxane), ^{1}H NMR ($CDCl_3$) δ 0 88 - 1 02 (12H, m, CH_3 Hyv and Val), 1 42 (9H, s, Boc), 2 2 (2H, m, H–β Hyv and H-β Val), 4 3 (1H, m, H-α Val), 4 81 (1H, d, H-α Hyv), 5 15 (2H, m, CH_2 Bzl), 7 31 (5H, m, C_6H_5 Bzl)

TFA.Val-D-Hyv-OBzl (11) (1 63 g, 4 mmol) prepared above was treated with trifluoroacetic acid (4 ml) for 30 min The mixture was triturated with diethyl ether and the TFA salt 11 of the resulting amino-free depsipeptide was obtained as a white powder (1 5 g, 89%), which was homogeneous according to TLC analysis (ethyl acetate / hexane 30 70; Rf 0 25), $[\alpha]^{20}_D$ +25° (c 2, MeOH) [lit. +24.5° (c 2, MeOH), hydrochloride][15c]

Boc-D-Val-Lac-Val-D-Hyv-OBzl (12) [15c] Diisopropylethylamine (1 2 ml, 6 8 mmol), and BOP reagent (1 45 g, 3 3 mmol) were added successively to a stirred solution of the trifluoroacetic salt **11** (1 26 g, 3 mmol) and the acid **10** (870 mg, 1 mmol) in methylene chloride (20 ml) Stirring was continued for 1 h The reaction mixture was then washed with 5% $KHSO_4$ (2x20 ml), water, 5% NaHCO3 (2x20 ml), and saturated brine The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue which was chromatographed on silicagel (ethyl acetate / hexane, 30 70) The tetradepsipeptide was isolated as a colorless oil (1 4 g, 80% yield), which was homogeneous according to TLC analysis (ethyl acetate / hexane 50 50, Rf 0 82), $[\alpha]^{20}_D$ -9 5° (c 2, EtOH), ^{1}H NMR ($CDCl_3$) δ 0 85 1 02 (18H, m, CH_3 Hyv, D-Val and Val), 1 4 (9H, s, Boc), 1 48 (3H, d, CH_3 Lac), 2 12 (1H, m, H-β D-Val), 2 25 (2H, m, H-β Hyv and Val), 4 12 (1H, m, H-α D-Val), 4 5 (1H, m, H-α Val), 5 05 (1H, d, H-α Hyv), 5 12 (2H, m, CH_2 Bzl), 5 28 (1H, m, H-α Lac), 7 35 (5H, m, C_6H_5 Bzl)

Boc-D-Val-Lac-Val-D-Hyv-OH (14) was prepared by Pd/C catalytic hydrogenation of Boc-D-Val-Lac-Val-D-Hyv-OBzl (12) (580 mg, 1 mmol) following the method described for the preparation of Boc-D-Val-Lac-OH (10), to give 450 mg (92 %) of a compound identical to that previously described in the literature [15c]

TFA.D-Val-Lac-Val-D-Hyv-OBzl (13) was prepared from Boc-D-Val-Lac-Val-D-Hyv-OBzl (12) (580 mg, 1 mmol) following the method described for deprotection of Boc-Val-D-Hyv-OBzl (9) $[\alpha]^{20}_D$ +29° (c 2, EtOH) [lit +29 5° (c 2, EtOH), hydrochloride][15c]

Boc-(D-Val-Lac-Val-D-Hyv)$_2$-OBzl (15) [15c] was prepared from TFA-D-Val-Lac-Val-D-Hyv-OBzl (13) and Boc-D-Val-Lac-Val-D-Hyv-OH (14), following the method described above for the preparation of Boc-D-Val-Lac-Val-D-Hyv-OBzl (12), to give 760 mg (80% yield) of a compound identical to that previously described in the literature, $[\alpha]^{20}_D$ -5° (c 2, EtOH) [lit -5° (c 2, EtOH)] [15c]

Boc-(D-Val-Lac-Val-D-Hyv)$_2$-OH (16) [15c] was prepared from Boc-(D-Val-Lac-Val-D-Hyv)$_2$-OBzl (15), following the method described above for the preparation of Boc-D-Val-Lac-Val-D-Hyv-OH (14), to give 610 mg (89% yield) of a compound identical to that previously described in the literature [15c]

Boc-(D-Val-Lac-Val-D-Hyv)$_3$-OBzl (17)[15c] was prepared from TFA.D-Val-Lac-Val-D-Hyv-OBzl (355 mg, 0 6 mmol) (13) and Boc-(D-Val-Lac-Val-D-Hyv)$_2$-OH (515 mg, 0 6 mmol) (16), following the method described for the preparation of Boc-D-Val-Lac-Val-D-Hyv-OBzl (12), to give 710 mg (90% yield) of a compound identical to that previously described in the literature, $[\alpha]^{20}_D$ -9° (c 1 4, EtOH) [lit -9 4° (c 1 4, EtOH)] [15c]

Valinomycin Boc-(D-Val-Lac-Val-D-Hyv)$_3$-OBzl was fully deprotected by the usual catalytic hydrogenation, followed by trifluoroacetic acid treatment to give 600 mg of TFA (D-Val-L-Lac-L-Val-D-Hyv)$_3$-OH (17) (90% yield), $[\alpha]^{20}_D$ +9° (c 1, $CHCl_3$) [lit +9 3° (c 1, $CHCl_3$), hydrochloride] [15c] BOP reagent (175 mg, 0 4 mmol) and TEA (0 6 ml) was added to a solution of 17 (400 mg, 0 32 mmol) in dichloromethane (50 ml), and the mixture was stirred for 48 h at room temperature After the usual workup the residue was chromatographied on a low-bar silicagel column (hexane / ethyl acetate, 80 20) Valinomycin was crystallized in nitromethane (107 mg) and was identical to a commercial sample and the valinomycin previously described in the literature [15c,23]

Acknowledgement.

We are indebted to SNPE-France for a generous gift of IPCC reagent and financial support. We thank Pr Aubagnac for mass spectra measurements

References

1-Abbreviations and symbols follow the recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature (Eur J Biochem 1984, 138, 9) In addition the following abbreviations are used **BOP** (1H-1,2,3-benzotriazol-1-yloxy)-tris(dimethylamino)-phosphonium hexafluorophosphate, **COMODD** 2,2'-carbonyl-bis-(3,5-dioxo-4-methyl-1,2,4 oxadiazolidine, **DIEA** diisopropylethylamine, **DMAP** 4-dimethylaminopyridine, **EDCI** 1-ethyl 3-[3-(dimethylamino)propyl]-carbodiimide hydrochloride, Hyv L-a-isovaleric acid, IPCC isopropenyl chlorocarbonate, Lac L-lactic acid

2-For a review, see. "The Peptides, Analysis, Synthesis, Biology", Gross, E , Meienhofer, J., Eds , Academic Press New-York, 1981, Vol 3, pp 101-136

3-(a) Schemyakin, M M *Angew Chem* **1960,** *72*, 342 (b) Shemyakin, M. M., Ovchinnikof, Y A., Ivanov, V T., Kiryususkin, A A *Tetrahedron* **1963**, *19*, 581 (c) Tsunoda, A *J Antibiot* **1975**, *28*, 332 (d) Frappier, F , Pais, M , Elsworth, J F , Grove, J F *Phytochemistry* **1978**, *17*, 545 (e) Remers, W A in "Chemistry of Antitumor Antibiotics" Wiley Press, New York, 1979, pp 1-62 (f) Manger, A B in "Topics in Antibiotic Chemistry" P G Sammes Ed., Halsted Press, New York, 1980, pp 229-293 (g) Olsen, R K in "Chemistry and Biochemistry of Amino acids, Peptides and Proteins", Weinstein B , Ed , Dekker, New york 1983, pp 1-33 (h) Zabriskie, T M , Klock, J A., Ireland, C M., Marcus, A H , Molinski, T F., Faulkner, D J , Xu, C , Clardy, J C *J Am Chem Soc* **1986,** *108*, 3123 (i) Rinehart, K L , Kishore, V , Bible, K C , Sakai, R , Sullins D W , Li, K -M *J Nat Prod* **1988**, *51*, 1

4-(a) Stewart, F H C *Aust J Chem* , **1967**, *20*, 365 (b) Wang, S S , Gisin B F., Winter, D P , Makofske, R , Kulesha, I D , Tzongraki, C , Meienhofer, J *J Org Chem* **1977**, *42*, 1286 (c) Ono, N., Yamada, T , Saito, T., Tanaka, K , Kaji, A *Bull Chem Soc Jpn* **1978**, *51*, 2401 (d) Bocchi, V , Casnati, G , Dossena, A , Marchelli, R *Synthesis* **1979**, 957 (e) Lerchen, H G , Kunz, H *Tetrahedron Lett* **1985**, *26*, 5257

5-(a) Neises, B , Steglich, W *Angew Chem , Int Ed Engl* **1978**, 4475 (b) Gilon, C, Klausner, Y , Hassner, A *Tetrahedron Lett* **1979**, 3811 (c) Dhaon, M K , Olsen, R K , Ramasami, K *J Org Chem* , **1982**, *47*, 1962 (d) Kim, S , Lee, J I , Kim, Y C *J Org Chem* , **1985**, *50*, 560

6-Gamet, J P., Jacquier, R., Verducci, J *Tetrahedron* **1984**, *40*, 1995

7-Greco, P A., Hon, Y S , Perez-Medrano, A *J Am Chem Soc* **1988**, *110*, 1630

8-Joum, P , Poncet, J., Dufour, M N., Pantaloni, A , Castro, B *J Org Chem* , **1989**, *54*, 617

9-(a) Jaouadi, M., Selve, C , Dormoy, J R , Castro, B *Bull Soc Chim Fr* **1984**, II-409 (b) Jaouadi, M , Selve, C., Dormoy, J R , Castro, B , Martinez, J *Tetrahedron Lett* , **1985**, *26*, 1721

10-Joum, P , Castro, B , Zeggaf, C , Pantaloni, A , Senet, J P , Lecolier, S , Sennyey, G *Tetrahedron Lett* **1987**, *28*, 1661

11-Meienhofer, J in "The Peptides, Analysis, Synthesis, Biology", Gross, E , Meienhofer, J , Eds, Academic Press, New-York, 1979, Vol 1, pp 263-314

12-Anderson, G W , Callahan, F M *J Am Chem Soc* **1960**, *82*, 3359

13-Widmer, U, *Synthesis* **1983**, 135

14-Grenouillat, D , Senet, J P , Sennyey, G *Tetrahedron Lett* **1987**, *28*, 5827

15-(a) Shemyakin, M M , Aldonova, N A , Vinogradova, E I , Feigina M -Y *Tetrahedron Lett* **1963**, 1921. (b) Gisin B F , Merrifield, R B., Tosteson, D C *J Am Chem Soc* **1969**, *91*, 2691 (c) Losse, G , Klengel, H *Tetrahedron* **1971**, *27*, 1423

16- Castro, B , Dormoy, J R , Evin, G , Selve, C *Tetrahedron Lett* **1975**, 1219

17-Park, W K., Soneby, R R , Bumpus, *Biochemistry* **1967**, *6*, 3458

18-Goodacre, J., Ponsford, R J , Stirling, I *Tetrahedron Lett* **1975**, 3609

19-Wünsch, E , Zwick, A *Hoppe Seyler's Z Physiol Chem* **1963**, *333*, 108

20-Migoshi, M *Bull Chem Soc Jap* **1973**, *46*, 1489

21-Martinez, J , Laur, J , Castro, B *Tetrahedron* **1985**, *41*, 739

22- Anderson, G W , Zimmerman, J E., Callahan, F M *J Am Chem Soc* **1964**, *86*, 1839

23- Ovchinnikov, Y A , Ivanov, V T *Tetrahedron* **1974**, *30*, 1871