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PEPTIDE BOND FORMATION USING AN ENZYME MIMICKING APPROACH[1]

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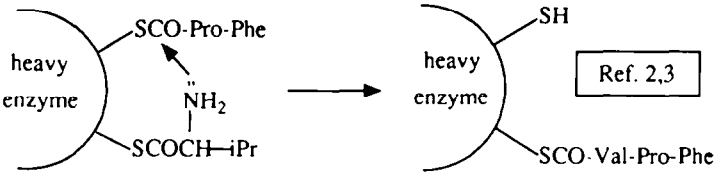
Abstract: *A man-made enzyme-model based on a concerted proton transfer step (bifunctional catalysis) which mimics the corresponding step in non-ribosomal peptide synthesis was developed. Important features of the model are the following: (a) a bifunctional acid-base catalyst for thiolester aminolysis rate acceleration, (b) two thiol-containing arms mimicking the "swinging arms" of the enzyme, and (c) symmetry elements so that the process can be iterated with consequent formation of the polypeptide chain. Peptide bond formation was obtained by intramolecularly catalyzed thiolester aminolysis to give* **5** *in 80% isolated yield (Scheme IV,V) and with at least a* 10^3*-fold rate acceleration in comparison with the corresponding non catalyzed process (***4*** → ***6****)(Scheme IV, Table I). The reaction is also 4-20 times faster than the analogous process* **4** → **6** *run in the presence of 0.1 M external catalyst (*Et_3N*-Bu*^{t}*COOH or 2-Pyridone). Important structural and reaction parameters are discussed. A second intramolecular aminolysis reaction gave tripeptide 8 in lower yield (35%) because of higher steric congestion in the transition state.*

During the 1970's the biosynthetic pathway occurring in some microorganisms and leading to peptides such as *Tyrocidine* and *Gramicidin-S* was completely elucidated.[2a] *Gramicidin-S* biosynthesis, for example, does not rely on the presence of ribosomes or tRNA. Two enzymes, a "light enzyme" (MW ca.100,000) and a "heavy enzyme" (MW ca.280,000) are involved, and the biosynthetic sequence appears to be much simpler and more primitive than the usual ribosomal protein biosynthesis.[2b,3a] The fundamental steps of the biosynthetic sequence are the following: (a) thiolesters are synthesized using ATP-activated α-aminoacids and thiol groups of the enzyme, (b) thiolesters are cleaved by enzyme-catalyzed intramolecular aminolysis with consequent formation of a peptide bond and of a free thiol group, and (c) this process is iterated with consequent formation of the polypeptide chain. This pathway is used by Nature for the production of relatively small peptides, consisting of about 10 residues. Here we report on a man-made enzyme-model which mimics this sequence of reactions.



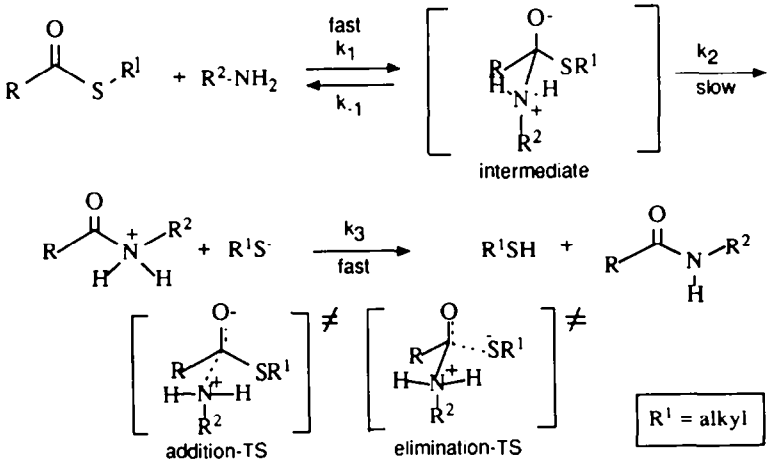
Previous attempts to mimic the thiolester mediated non-ribosomal peptide synthesis[4,5] substantially differ from the work reported here. In particular the catalytic step in Koga's approach is thiolester formation by thiolysis of a terminal p-nitrobenzoate of an aminoacid-$NH_3^+Br^-$ group complexed to a pendant crown ether, while the intramolecular aminolysis rate (S → N acyl transfer) is enhanced by standard buffering with pivalic

acid and triethylamine in benzene (*vide infra*).[5]

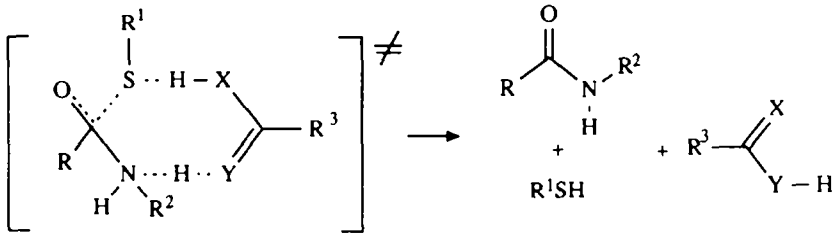
In our work thiolesters are easily synthesized using standard chemistry involving carboxylic acid activation through mixed anhydrides (e.g. DCC,4-PP; DPPA; DEPC; etc.)[6] in a way which is quite similar to biosynthetic ATP activation. Our efforts are directed toward catalysis of the intramolecular thiolester aminolysis step, which is the key reaction for peptide bond formation.[7]

The mechanism and kinetics of ester and thiolester aminolyses have been studied in some detail.[8] On the bases of those results reported in the literature[8b,c,d,e] the reaction mechanism can be tentatively described as shown in Scheme I. In most cases, when R^1 is alkyl or a weakly electron-withdrawing group, the rate determining step is thiol elimination and the reaction appears to be controlled by the elimination-TS (see Scheme I). Although thiolesters are high energy substrates and thiolester aminolysis is a thermodynamically favored process, the reaction is very sluggish in apolar solvents (a rate constant of 1.5×10^{-5} $sec^{-1}M^{-1}$ was reported for a simple intermolecular case in benzene).[5d] One way to increase the reaction rate is to decrease the elimination activation energy by changing R^1 from an alkyl group to a strongly electron-withdrawing group (e.g. p-nitrophenyl). Other ways to catalyze the process are by general acid-base catalysis in protic solvents (water),[8a] or by concerted proton-transfer at the TS level from the ammonium cation to the sulfide anion.[8i] A concerted proton-transfer assistance through an eight-membered ring (bifunctional catalysis, see Scheme II) has been used to catalyze several alkyl thiolester inter- and intramolecular aminolyses.[4,5,9] The most widely used bifunctional catalysts are bicarbonate, the monoanion of phosphate, substituted phosphonates, methyl arsonate in aqueous solutions, and 2-hydroxypyridines (e.g. 2-pyridone) or carboxylic acids (e.g. pivalic acid, acetic acid) in aprotic solvents.[3b,8h,10,11]

Scheme I



Scheme II



A typical thiolester aminolysis is usually carried out in benzene at room temperature in the presence of 0.15 M pivalic acid and 0.15 M Et_3N. Rate constants are usually around $2\text{-}6x10^{-5}$ sec^{-1} for the intramolecular cases.[5]

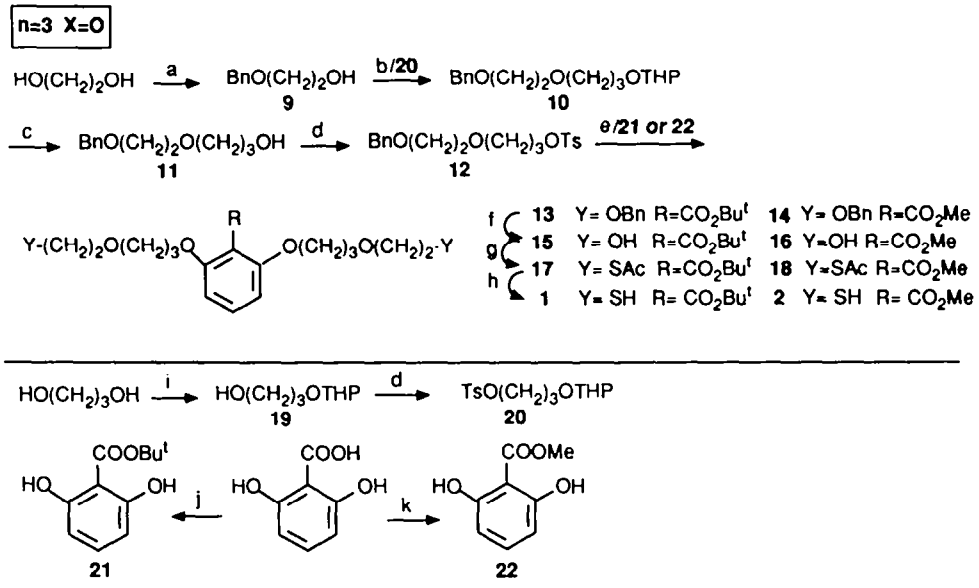
Important features of the bifunctionally catalyzed aminolyses are the following : (a) Et_3N alone has little or no catalytic activity,[8h] (b) relatively weak carboxylic acids such as pivalic and acetic acid strongly catalyze the aminolysis reaction,[5d,8h] (c) α-pyridone is almost as effective as acetic acid,[8h] (d) with increasing concentrations of acid (>0.1 M) the rate acceleration decreases because the nucleophilic amine is protonated to a greater extent, and therefore is less reactive,[5d,8h] (e) the solution can be buffered by added Et_3N; however, increasing amounts of pivalic acid and Et_3N shift the equilibrium towards the inactive triethylammonium pivalate and therefore only a 3.4 fold rate acceleration (dependent on the amount of *free* pivalic acid) is achieved upon increasing the acid and amine concentrations 20-fold (from 0.005 M to 0.1 M).[5d]

It is evident from these data that the rate acceleration obtained with 0.1 M pivalic acid and 0.1 M Et_3N cannot be pushed much further.

We have developed a bifunctional catalyst bearing pendant thiol groups and possessing the following features: (a) a bifunctional acid-base catalyst for promoting thiolester aminolysis rate acceleration, (b) two thiol-containing arms which mimic the "swinging arms" of the enzyme,[1,2,3] and (c) C_2-symmetry so that the process can be iterated with consequent formation of a polypeptide chain.

Starting from commercially available 2,6-dihydroxybenzoic acid, dithiols **1** and **2** were easily synthesized (Scheme III). Monoacylation with Z-Gly (using DCC/4-PP[6] or DEPC[6] activation, 50-55%) and acylation again with BOC-Ala (DPPA/DMF,[6] 90-95%) gave compounds **3** and **4** (Scheme IV). Transacylation was effected by removal of the BOC and the t-butyl ester groups of **3** (1:1 TFA-CH_2Cl_2, 0°C, 100%), evaporation of solvent, and addition of Et_3N (1.5 mol.equiv.)(to neutralize the TFA-salt) to a benzene solution of the intermediate under high dilution ($1x10^{-3}$ M)(Scheme IV).

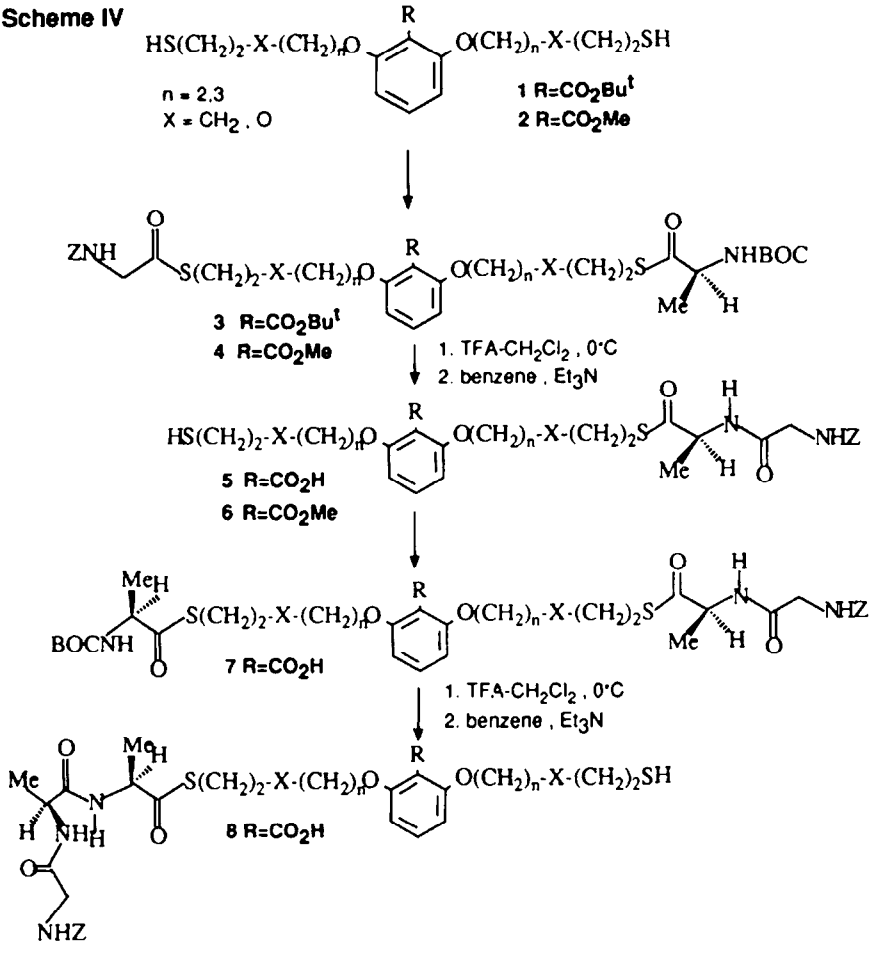
Scheme III. Synthesis of dithiols **1** and **2**.



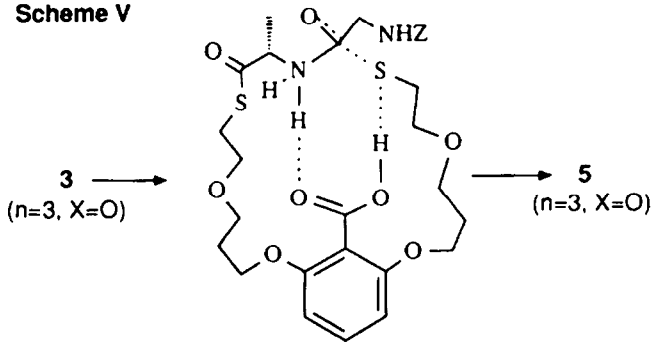
Reagents and average yields:

a) NaH, BnBr, THF, Bu_4NI cat. (50%) b) Bu_4NHSO_4, 50% NaOH in H_2O, toluene (84%) c) MeOH, PPTS (95%) d) TsCl, C_5H_5N, 4-DMAP cat.(85%) e) Cs_2CO_3, DMF (60-91%) f) H_2, Pd-C, MeOH (99%) g) AcSH, DEAD, PPh_3, THF (74-90%) h) MeONa, MeOH (85%) i) DHP, CH_2Cl_2, PTSA cat. (50%) j) $Me_2NCH(OBu^t)_2$, benzene (65%) k) MeOH, H_2SO_4 (65%) l) NaH, THF, TBDMSCl (70%) m) TBAF, THF (95%)

Scheme IV



Scheme V



Peptide bond formation is proposed to occur with catalysis by rapid proton transfer through an eight-membered ring *via* the properly oriented carboxylic acid (Scheme V).[12] Experiments with n=3, R=CO_2H gave dipeptide **5** (80% isolated yield) with rate constants of 8×10^{-5}sec^{-1} (X=O, $t_{1/2}$= 2 h and 25 min) and 5×10^{-5} sec^{-1} (X=CH_2, $t_{1/2}$= 3 h and 50 min), and with ***at least a 10^3-fold rate acceleration*** in comparison with the

corresponding noncatalyzed processes [**4** → **6**, n=3, X=O or CH_2, R=CO_2Me, ***no reaction product detected after 6 months*** ($k < 5x10^{-8}$ sec^{-1})]. This acceleration is still relatively small, compared to those reported in other cases (e.g.$1.5x10^{5}$),[13] but shows the feasibility of the process. The reactions are also 4-8 times faster than the analogous processes **4** → **6** (n=3, R=CO_2Me) run in the presence of 0.1 M pivalic acid and 0.1 M Et_3N which are characterized by rate constants of $2x10^{-5}$ sec^{-1}(X=O) and $6.2x10^{-6}$ sec^{-1} (X=CH_2), and are approximately 20 times faster than the reactions catalyzed by 0.1 M 2-Pyridone (Table I).

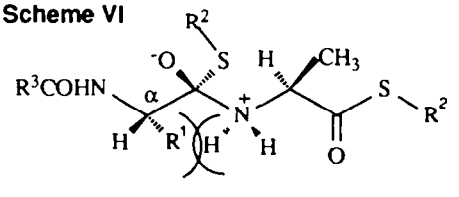
Table I. Intramolecular aminolyses in benzene (10^{-3}M).

Rxn	Product	n	X	k(sec^{-1})	$t_{1/2}$(min)	External catalyst [M]
	5	3	O	$8x10^{-5}$	145	--
	5	3	CH_2	$5x10^{-5}$	230	--
	6	3	O	$<5x10^{-8}$	--	--
	6	3	CH_2	$<5x10^{-8}$	--	--
	6	3	O	$2x10^{-5}$	575	Bu^tCOOH [0.1] Et_3N [0.1]
	6	3	O	$3.4x10^{-6}$	3300	2-Pyridone [0.1]
	6	3	CH_2	$6.2x10^{-6}$	1860	Bu^tCOOH [0.1] Et_3N [0.1]
	6	3	CH_2	$2.9x10^{-6}$	3900	2-Pyridone [0.1]
	5	2	O	$7x10^{-5}$	165	--

Important features of these transformations include the following: (a) bifunctional catalysis is inhibited by more polar solvents (e.g. CH_2Cl_2, CH_3CN, DMF, DMSO), (b) weaker (Pyridine, 2,6-Lutidine) or stronger (DBU) amines are less effective than Et_3N, (c) more than 2 or less than 1 mol. equiv. of Et_3N tend to slow down the reaction, because of carboxylate formation (more than 2) or incomplete neutralization of the TFA-salt (less than 1), (d) the presence of oxygen in the arms makes a difference: there is a 1.6-3.2 fold rate acceleration on going from n=3, X=CH_2 to n=3, X=O, (e) CPK models suggest that the 21- and 23-membered macrocyclic transition states, corresponding to n=2,3 (Scheme IV, V), allow for the best orientation for the bifunctional catalysis, and (f) no significant rate difference was observed between the n=2 and n=3 series (e.g. n=2, X=O, R=CO_2H, $k=7x10^{-5}$ sec^{-1}).

A new BOC-protected aminoacid (e.g. BOC-Ala) can then be added (DPPA/DMF,85%) to give **7** (n=3, X=O). Unfortunately the second intramolecular aminolysis (Scheme IV) appears to be complicated by unfavorable steric hindrance at the α-carbon of the acyl group (CHMe vs. CH_2 of the first aminolysis, Scheme VI),[14] and formation of the desired tripeptide **8** (n=3, X=O) proceeds in low yield (35%); dipeptide **5** and Ala-Ala-S-(Spacer)-S-Ala-Gly-Z, derived from intermolecular aminolysis, are also isolated. We are currently working on a new macrocyclic model system with reduced conformational freedom of the two arms which should give higher rate accelerations and reduce the amount of side products formed.

Scheme VI



R^1= H (80% yield)
R^1= Me (35% yield)

During the past two decades there has been an enormous interest in developing chemical models of enzymatic catalysis. The high efficiency of enzymatic catalysis has been generally attributed to fundamental chemical mechanisms which operate under favorable conditions present in the enzyme-substrate complex. Several of these - general acid, general base, nucleophilic, and bifunctional catalysis - appear to be particularly common to enzymes. Models of each have been studied with the goal of approximating the catalytic efficiency observed in enzymes.[15] We have reported here a chemical model for peptide synthesis based on a concerted proton transfer (bifunctional catalysis).

Experimental

^{1}H NMR spectra were recorded with a Bruker AC-200 instrument in the FT mode with tetramethylsilane as internal standard. IR spectra were recorded with a Perkin-Elmer 681 spectrophotometer. Silica gel 60 F_{254} plates (Merck) were used for analytical TLC, 270-400 mesh silica gel (Merck) for flash chromatography. "Dry" solvents were distilled under N_2 just before use: tetrahydrofuran (THF), benzene, toluene and diethyl ether were distilled from sodium metal (THF and diethyl ether were distilled in the presence of benzophenone); dimethyl formamide (DMF) and methylene chloride from CaH_2. All reactions employing dry solvents were run under a nitrogen (from liquid N_2) atmosphere. The organic solvents were dried over sodium sulfate (Na_2SO_4) and evaporated under reduced pressure.

Note: all compounds described below had microanalyses which agreed with calculated values within ± 0.3 % (C,H). High resolution MS spectra (FAB) were obtained for all the compounds described below, confirming the molecular weights. All compounds described below are oils, unless otherwise stated (m.p. reported). Routine IR spectra were recorded for all the compounds described below, and showed the characteristic absorbances of the various functional groups [e.g. compound **3** *(**n=3, X=O**). IR ($CHCl_3$) selected values: 3440 (ν_{N-H}), 3030-3000 (ν_{C-H}), 1720-1680 ($\nu_{C=O}$ ArCOOR 1715, $\nu_{C=O}$ ROCONHR 1700, $\nu_{C=O}$ RCOSR 1690), 1595 ($\nu_{C=C}$), 1530-20 (ν_{C-N} + δ_{N-H}), 1250-1150 (ν_{C-O}), 1100 (ν_{C-O}) cm^{-1}]. Characterization reported below is based on ^{1}NMR spectroscopy.*

Monoprotected ethylene glycol **9**. A suspension of NaH (1.224 g, 51.0 mmol) was treated with ethylene glycol (2.80 g, 51.0 mmol) and stirred for 1.5 hr at room temperature. To this suspension, heated to 45°C, benzyl bromide (4.361 g, 25.5 mmol) and n-Bu_4NI (94 mg, 0.25 mmol) were added. After stirring for 3 hr at 45°C, the reaction mixture was diluted with ethyl acetate (100 ml) and washed with a saturated NH_4Cl aq. solution. The organic phase was dried and the solvent evaporated. The crude product was purified by flash chromatography (n-hexane-ethyl acetate 55:45) to give the monoprotected ethylene glycol **9** as an oil in 50% yield. ^{1}H NMR ($CDCl_3/D_2O$) δ: 3.62 (2H, t, J=9.5Hz), 3.77 (2H, t, J=4.39Hz), 4.58 (2H, s), 7.34 (5H, bs).

Ether **10**. A solution of tosylate **20** (1.275 g, 4.057 mmol) and alcohol **9** (0.641 g, 4.057 mmol) in toluene (1.76 ml) was treated with NaOH (1.622 g, 40.570 mmol), water (1.622 ml) and n-Bu_4NHSO_4 (0.137 g, 0.406 mmol). The reaction mixture was stirred for 3 hr at 80°C, and then cooled to room temperature and treated with a saturated aq. NH_4Cl solution (75 ml). The aqueous solution was extracted with ethyl acetate (3x50 ml), the organic extracts were dried and the solvent evaporated. The crude product was purified by flash chromatography (n-hexane-ethyl acetate 7:3) to give ether **10** in 84% yield. (Modified procedure from ref. 16). ^{1}H NMR ($CDCl_3$) δ: 1.51-1.80 (6H, m), 1.86-1.98 (2H, m), 3.45-3.64 (8H, m), 3.77-3.93 (2H, m), 4.61 (3H, s), 7.34 (5H, bs).

Alcohol **11**. A solution of ether **10** (2.759 g, 9.197 mmol) in methanol (23 ml) was treated with pyridinium toluene-4-sulfonate (PPTS) (0.391 g, 1.56 mmol) and then refluxed for 3 hr. The solvent was evaporated and the crude product was purified by flash chromatography (n-hexane-ethyl acetate 45:55) to give alcohol **11** in 95% yield. ^{1}H NMR ($CDCl_3/D_2O$) δ: 1.68-1.96 (2H, m), 3.59-3.82 (8H, m) 4.58 (2H, s), 7.34 (5H, bs).

General procedure for the synthesis of Tosylates **12, 20, 24, 32.**

Tosylate **12**. A solution of alcohol **11** (0.648 g, 3.086 mmol) in dry pyridine (6.20 ml) was treated with tosyl chloride (0.764 mg, 4.011 mmol) and 4-dimethylaminopyridine (DMAP) and stirred for 3 hr at 0°C. The reaction

mixture was then diluted with ethyl acetate and washed with 1 N HCl. The organic layer was dried and the solvent evaporated; the crude product was purified by flash chromatography (n-hexane-ethyl acetate 7:3) to give tosylate **12** in 85% yield. ^{1}H NMR ($CDCl_3$) δ: 1.87-2.08 (2H, m), 2.41 (3H, s), 3.41-3.59 (6H, m), 4.17(2H, t, J=6.67Hz), 4.54 (2H, s), 7.34 (2H, d, J=8.35), 7.34 (5H, bs), 7.78 (2H, d, J=8.35).

Tosylate **20**. Purified by flash chromatography (n-hexane-ethyl acetate 7:3). Yield: 70%. ^{1}H NMR ($CDCl_3$) δ: 1.41-1.70 (6H, m), 1.86-1.94 (2H, m), 2.41 (3H, s), 3.32-3.50 (2H, m), 3.70-3.82 (2H, m), 4.26 (2H, t, J=5.85Hz), 4.40-4.49 (1H, m), 7.36 (2H, d, J=8.35Hz), 7.80 (2H, d, J=8.35Hz).

Tosylate **24**. Purified by flash chromatography (n-hexane-ethyl acetate 9:1). Yield: 85%. ^{1}H NMR ($CDCl_3$) δ: 0.04 (6H, s), 0.89 (9H, s), 1.25-1.51 (6H, m), 1.62-1.71 (2H, m), 2.41 (3H, s), 3.57 (2H, t, J=6.50Hz), 4.02 (2H, t, J=6.50Hz), 7.36 (2H, d, J=8.35Hz), 7.78 (2H, d, J=8.35Hz).

Tosylate **32**. Purified by flash chromatography (n-hexane-ethyl acetate 85:15). Yield: 79%. ^{1}H NMR ($CDCl_3$) δ: 0.05 (6H, s), 0.90 (9H, s), 2.41 (3H, s), 3.50-3.80 (6H, m), 4.30 (2H, t, J=6.30Hz), 7.36 (2H, d, J=8.35Hz), 7.80 (2H, d, J=8.35Hz).

General procedure for the synthesis of Diphenolic ethers **13, 14, 25, 26, 33.**

Dibenzylderivate **13**. A solution of diphenol **21** (0.220 g, 1.046 mmol) in dry DMF (8 ml) was treated with cesium carbonate (Cs_2CO_3) (1.022 g, 3.138 mmol); after stirring for 30 min, a solution of tosylate **20** (0.800 g, 2.197 mmol) in dry DMF (2 ml) was added. The reaction mixture was stirred for 10 hr at room temperature, then diluted with ethyl acetate (50 ml) and washed with a saturated aq. NH_4Cl solution. The organic extracts were dried, evaporated and the crude product was purified by flash chromatography (n-hexane-ethyl acetate 65:35) to give **13** in 78% yield. (Modified procedure from ref. 17). ^{1}H NMR ($CDCl_3$) δ: 1.58 (9H, s), 2.02-2.17 (4H, m), 3.61-3.70 (12H, m), 4.09 (4H, t, J=6.67Hz), 4.58 (4H, s), 6.53 (2H, d, J=7.75Hz), 7.18 (1H, t, J=7.75Hz), 7.37 (10H, bs).

Dibenzylderivate **14**. Purified by flash chromatography (n-hexane-ethyl acetate 6:4). Yield: 72%. ^{1}H NMR ($CDCl_3$) δ: 2.02-2.19 (4H, m), 3.60-3.70 (12H, m), 3.88 (3H, s), 4.08-4.16 (4H, m), 6.56 (2H, d, J=7.95Hz), 7.22 (1H, T, J=7.95Hz), 7.37 (10H, bs).

Protected diol **25**. Purified by flash chromatography (n-hexane-ethyl acetate 9:1). Yield: 84%. ^{1}H NMR ($CDCl_3$) δ: 0.10 (12H, s), 0.94 (18H, s), 1.35-1.58 (12H, m), 1.56 (9H, s), 1.68-1.83 (4H, m), 3.60 (4H, t, J=6.50Hz), 3.98 (4H, t, J=6.50Hz), 6.49 (2H, d, J=7.75Hz), 7.18 (1H, t, J=7.75Hz).

Protected diol **26**. Purified by flash chromatography (n-hexane-ethyl acetate 9:1). Yield: 60%. ^{1}H NMR ($CDCl_3$) δ: 0.10 (12H, s), 0.94 (18H, s), 1.32-1.60 (12H, m), 1.56 (9H, s), 1.68-1.83 (4H, m), 3.60 (4H, t, J=6.50Hz), 3.98 (4H, t, 6.50Hz), 6.49 (2H, d, J=7.75Hz), 7.18 (1H, t, J=7.75Hz).

Protected diol **33**. Purified by flash chromatography (n-hexane-ethyl acetate 8:2). Yield: 91%. ^{1}H NMR ($CDCl_3$) δ: 0.09 (12H, s), 0.90 (18H, s), 1.52 (9H, s), 3.48-3.89 (12H, m), 4.08 (4H, t, J=6.09Hz), 6.51 (2H, d, J=7.90Hz), 7.19 (1H, t, J=7.90Hz).

General procedure for the synthesis of diols **15** and **16.**

Diol **15**. A suspension of palladium on activated charcoal (10% Pd) (95 mg, 0.088 mmol) in MeOH (1 ml) was treated with a solution of **13** (264 mg, 0.444 mmol) in MeOH (3 ml). The reaction mixture was stirred under hydrogen atmosphere for 1 hr; the mixture was filtered on a celite pad and the solvent evaporated. The crude product was purified by flash chromatography (methylene chloride-methanol 95:5) to give **15** in 99% yield. ^{1}H NMR ($CDCl_3/D_2O$) δ: 1.58 (9H, s), 2.01-2.19 (4H, m), 3.52 (4H, t, J=5.50Hz), 3.53-3.71 (8H, m), 4.05-4.17 (4H, m), 6.53 (2H,d, J=7.75Hz), 7.20 (1H, t, J=7.75Hz).

Diol **16**. Purified by flash chromatography (ethyl acetate-methanol 98:2). Yield: 99%. ^{1}H NMR ($CDCl_3/D_2O$) δ: 2.02-2.18 (4H, m), 3.54-3.72 (12H, m), 3.91(3H, s), 4.11 (4H, t, J=5.95Hz), 6.56 (2H, d, J=7.95Hz), 7.22 (1H, t, J=7.95Hz).

General procedure for the synthesis of dithiolesters **17, 18, 29, 30, 35.**

Dithiolester **17**. To a solution of PPh_3 (1.785 g, 2.72 mmol) in dry THF (8 ml) diethyl azodicarboxylate (DEAD) (1.067 ml, 6.81 mmol) was slowly added at 0°C. After stirring for 20 min, the reaction mixture was treated with a solution of diol **15** (1.128 g, 2.72 mmol) and thioacetic acid (AcSH) (0.484 ml, 6.81 mmol) in dry THF (5 ml). The resulting solution was stirred for 1 hr at 0°C and at room temperature for 2 hr. The solvent was evaporated and the crude product was purified by flash chromatography (n-hexane-ethyl acetate 7:3) to give **17** in 84% yield. (Modified procedure from ref. 18). ^{1}H NMR ($CDCl_3$) δ: 1.58 (9H, s), 2.03(4H, t, J=6.50Hz), 2.32 (6H, s), 3.08 (4H, t, J=6.80Hz), 3.57 (4H, t, J=6.50Hz), 3.61 (4H, t, J=6.80Hz), 4.09 (4H, t, J=6.50Hz), 6.52 (2H, d, J=7.75Hz), 7.20 (1H, t, J=7.75Hz).

Dithiolester **18**. Purified by flash chromatography (n-hexane-ethyl acetate 65:35). Yield: 87%. ^{1}H NMR ($CDCl_3$) δ: 2.03 (4H, t, J=6.50Hz), 2.37 (6H, s), 3.09 (4H, t, J=6.80Hz), 3.54-3.68 (8H, m), 3.88 (3H, s), 4.04 (4H, t, J=6.50 Hz), 6.58 (2H, d, J=7.95Hz), 7.22 (1H, t, J=7.95Hz).

Dithiolester **29**. Purified by flash chromatography (n-hexane-dietyl ether 7:3). Yield: 90%. ^{1}H NMR ($CDCl_3$) δ: 1.47-1.78 (16H, m), 1.51 (9H, s), 2.32 (6H, s), 2.90 (4H,t, J=6.50Hz), 3.98 (4H, t, J=6.50Hz), 6.49 (2H, d,

J=7.75Hz), 7.20 (1H, t, J=7.75Hz).

Dithiolester **30**. Purified by flash chromatography (n-hexane-ethyl acetate 8:2). Yield: 74%. ^{1}H NMR ($CDCl_3$) δ: 1.37-1.80 (16H, m), 2.32 (6H, s), 2.88 (4H, t, J=6.50Hz), 3.90 (3H, s), 4.08 (4H, t, J=6.50Hz), 6.52 (2H, d, J=7.98Hz), 7.22 (1H, t, J=7.98Hz).

Dithiolester **35**. Purified by flash chromatography (n-hexane-ethyl acetate 6:4). Yield: 82%. ^{1}H NMR ($CDCl_3$) δ: 1.59 (9H, s), 2.36 (6H, s), 3.11 (4H, t, J=6.50Hz), 3.68 (4H, t, J=6.50Hz), 3.81 (4H, t, J=5.40Hz), 4.14 (4H, t, J=5.40Hz), 6.54 (2H, d, J=7.90Hz), 7.22 (1H, t, J=7.90Hz).

General procedure for the hydrolysis of the dithiolesters to give dithiols **1** and **2**.

Dithiol **1 (n=3, X=O)**. A solution of dithiolester **17** (1.207 g, 2.277 mmol) in dry MeOH (2.5 ml) was added to a stirred suspension of MeONa (0.492 g, 9.110 mmol) in dry MeOH (10 ml) at room temperature. After 5 min the reaction mixture was treated with glacial acetic acid (520 μl) and the solvent evaporated. The crude product was purified by flash chromatography (n-hexane-ethyl acetate 65:35) to give dithiol **1** (**n=3, X=O**) in 85% yield. ^{1}H NMR ($CDCl_3$) δ: 1.56 (2H, t, J=5.82Hz), 1.58 (9H, s), 1.92 (4H, tt, J=6.50,6.80Hz), 2.68 (4H, dt, J=5.82,6.80Hz), 3.59 (4H, t, J=6.80Hz), 3.62 (4H, t, J=6.50Hz), 4.11 (4H, t, J=6.50Hz), 6.52 (2H, d, J=7.75Hz), 7.20 (1H, t, J=7.75Hz).

Dithiol **2 (n=3, X=O)**. Purified by flash chromatography (n-hexane-ethyl acetate 65:35). Yield: 83% ^{1}H NMR ($CDCl_3$) δ: 1.50-1.56 (2H, m), 2.06 (4H, tt, J=6.50,6.77Hz), 2.71 (4H,dt, J=6.77,5.90Hz), 3.54-3.65 (8H, m), 3.90 (3H, s), 4.12 (4H, t, J=6.50Hz), 6.58 (2H, d, J=7.95Hz), 7.22 (1H, t, J=7.95Hz).

Dithiol **1 (n=3, X=CH_2)**. Purified by flash chromatography (n-hexane-ethyl acetate 85:15). Yield: 87%. ^{1}H NMR ($CDCl_3$) δ: 1.31 (2H, t, J=7.10), 1.44-1.81 (16H, m), 1.53 (9H, s), 2.51 (4H, dt, J=7.10, 6.50Hz), 3.98 (4H, t, J=6.50Hz), 6.49 (2H, d, J=7.75Hz), 7.18 (1H, t, J=7.75Hz).

Dithiol **2 (n=3, X=CH_2)**. Purified by flash chromatography (n-hexane-ethyl acetate 85:15). Yield: 88%. ^{1}H NMR ($CDCl_3$) δ: 1.40-1.82 (16H, m), 2.51 (4H, dt, J=7.10,6.50Hz), 3.90 (3H, s), 3.98 (4H, t, J=6.50Hz), 6.52 (2H, d, J=7.98Hz), 7.22 (1H, t, J=7.98Hz).

Dithiol **1 (n=2, X=O)**. Purified by flash chromatography (n-hexane-ethyl acetate 65:35). Yield: 79%. ^{1}H NMR ($CDCl_3$) δ: 1.48 (9H, s), 1.53 (2H, t, J=6.54Hz), 2.71 (4H, dt, J=6.54, 6.50Hz), 3.70 (4H, t, J=6.50Hz), 3.82 (4H, t, J=5.40Hz), 4.18 (4H, t, J=6.50Hz), 6.54 (2H, d, J=7.90Hz), 7.22 (1H,t, J=7.90Hz).

Alcohol **19**. A solution of 1,3-propanediol (4.75 ml, 65.7 mmol) in methylene chloride was treated with 3,4-dihydro-2H-pyran (DHP) (3.0 ml, 32.85 mmol) and a catalytic amount of toluene-4-sulfonic acid monohydrate (PTSA) (63 mg, 0.33 mmol). The reaction mixture was stirred at room temperature for 1 hr and then triethylamine (150 μl) was added; the solvent was evaporated and the crude product was purified by flash chromatography (n-hexane-ethyl acetate 4:6) to give **19** in 50% yield. ^{1}H NMR ($CDCl_3/D_2O$) δ: 1.42-1.73 (4H, m), 1.75-1.90 (4H, m), 3.45-3.67 (2H, m), 3.72-3.95 (4H, m), 4.54-4.64 (1H, m).

Diphenol **21**. A suspension of 2,6-dihydroxybenzoic acid (1.00 g, 6.49 mmol) in dry benzene (10 ml) was heated to 80°C and N,N-dimethylformamide di-tert-butyl acetal (3.11 ml, 12.99 mmol) was added dropwise during 10 min. After stirring at 80°C for 10 min the reaction mixture was diluted with ethyl acetate (25 ml) and washed with 1 N HCl. The organic extracts were dried and the solvent evaporated; the crude product was purified by flash chromatography (n-hexane-ethyl acetate 95:5) to give **21** in 65% yield. (Modified procedure from ref. 19). ^{1}H NMR ($CDCl_3/D_2O$) δ: 1.71 (9H, s), 6.48 (2H, d, J=7.55Hz), 7.28 (1H, t, J=7.55Hz).

Diphenol **22**. A solution of 2,6-dihydroxybenzoic acid (5 g, 32.40 mmol) in methanol (13 ml) was refluxed for 36 hr in the presence of concentrated H_2SO_4 (1.30 ml). The solvent was evaporated and the crude product was recrystallized from n-hexane to give **22** in 65% yield (m.p. 57-58°C). ^{1}H NMR ($CDCl_3/D_2O$) δ: 4.10 (3H, s), 6.47 (2H, d, J=8.04Hz), 7.24 (1H, t, J=8.04Hz).

General procedure for the synthesis of alcohols **23** and **31**.

Alcohol **23**. Sodium hydride (NaH 55% in oil) (1.846 g, 42.31 mmol) was suspended in dry THF (80 ml) after being washed with n-hexane. 1,6-Hexanediol (5.0 g, 42.31 mmol) was added to this mixture at 0°C and stirred at room temperature for 90 min; *tert*-butyldimethylsilyl chloride (6.377 g, 42.31 mmol) was then added, and vigorous stirring was continued for 90 min at room temperature. The reaction mixture was poured into diethyl ether (300 ml), washed with 10% aq. K_2CO_3 (100 ml) and brine (100 ml). The organic layer was dried and evaporated. The crude product was purified by flash chromatography (n-hexane-ethyl acetate 7:3) to give **23** in 70% yield. (Modified procedure from ref. 20). ^{1}H NMR ($CDCl_3/D_2O$) δ: 0.10 (6H, s), 0.90 (9H, s), 1.19-1.81 (8H, m), 3.57-3.81 (4H, m).

Alcohol **31**. Purified by flash chromatography (n-hexane-ethyl acetate 7:3). Yield: 75%. ^{1}H NMR ($CDCl_3/D_2O$) δ: 0.10 (6H, s), 0.90 (9H, s), 3.53-3.85 (8H, m).

General procedure for the synthesis of diols **27, 28, 34.**

Diol **27**. Compound **25** (0.607 g, 0.95 mmol) was dissolved in a 1.0 *M* solution of tetrabutylammonium fluoride (TBAF) in THF (3.8 ml). The reaction mixture was stirred for 1 hr at room temperature and then diluted with ethyl acetate (40 ml) and washed with water (10 ml); the organic extracts were dried and evaporated. The crude product was purified by flash chromatography (n-hexane-ethyl acetate 3:7) to give **27** in 99% yield. ^{1}H NMR ($CDCl_3/D_2O$) δ: 1.39-1.94 (16H, m), 1.51 (9H, s), 3.67 (4H, t, J=6.50Hz), 3.99 (4H, t, J=6.50Hz), 6.52 (2H, d, J=7.75Hz), 7.20 (1H, t, J=7.75Hz).

Diol **28**. Purified by flash chromatography (n-hexane-ethyl acetate 15:85). Yield: 99%. ^{1}H NMR ($CDCl_3/D_2O$) δ: 1.37-1.77 (16H, m), 3.57 (4H, t, J=6.50Hz), 3.90 (3H, s), 3.99 (4H, t, J=6.50Hz), 6.50 (2H, d, J=8.04Hz), 7.22 (1H, t, J=8.04Hz).

Diol **34**. Purified by flash chromatography (ethyl acetate-MeOH 97:3). Yield: 76%. ^{1}H NMR ($CDCl_3/D_2O$) δ: 1.52 (9H, s), 3.51-3.72 (8H, m), 3.82 (4H,t, J=5.40Hz), 4.19 (4H, t, J=5.40Hz), 6.57 (2H, d, J=7.90Hz), 7.22 (1H, t, J=7.90Hz).

General procedure for the synthesis of Dithiolester **3** and **4**.

Dithiolester **3 (n=3, X=O)**. Dithiol **1 (n=3, X=O)** (436 mg, 0.966 mmol) was added to a stirred solution of Z-Gly (202 mg, 0.966 mmol) and DCC (199 mg, 0.966 mmol) in dry methylene chloride (15 ml) at room temperature. After 5 min 4-Pyrrolidinopyridine (4-PP) (21.5 mg, 0.145 mmol) was added and the solution stirred for 4 hr. The solvent was then filtered and evaporated; the crude product was purified by flash chromatography (n-hexane-ethyl acetate 1:1) to give the **mono-Z-Gly derivative** in 50% yield. mono-Z-Gly derivative **(n=3, X=O, R=COOtBu)** : ^{1}H NMR ($CDCl_3$) δ: 1.59 (9H, s), 1.95-2.04 (4H, m), 2.64 (2H, dt, J=6.77,6.50Hz), 3.12 (2H, t, 6.50Hz), 3.51-3.68 (8H, m), 4.01-4.18 (6H, m), 5.18 (2H, s), 6.53 (2H, dd, J=7.15Hz), 7.21 (1H, T,J=7.15Hz), 7.48 (5H, bs). A solution of this compound (438 mg, 0.688 mmol) in dry DMF (7 ml) was treated with diphenylphosphoryl azide (DPPA) (253 µl, 1.170 mmol), Ala-Boc (195 mg, 1.030 mmol) and triethylamine (144 µl, 1.030 mmol) at 0°C. After stirring for 2 hr the solution was diluted with ethyl acetate (50 ml) and washed with brine (25 ml); the organic extracts were dried and evaporated. The crude product was purified by flash chromatography (n-hexane-ethyl acetate 55:45) to give dithiolester **3 (n=3, X=O)** in 92% yield. (Modified procedure from ref. 21). ^{1}H NMR ($CDCl_3$) δ: 1.36 (3H, d, J=6.80Hz), 1.46 (9H, s), 1.57 (9H, s), 1.93-2.04 (4H, m), 3.03-3.14 (4H, m), 3.45-3.52 (8H, m), 4.01-4.11 (6H, m), 4.29-4.41 (1H, m), 5.18 (2H, s), 6.52 (2H, dd, J=7.15Hz), 7.21 (1H, t, J=7.15Hz), 7.48 (5H,bs).

In all the reaction sequences leading to the formation of dithiolesters **3** and **4**, mono-Z-Gly derivatives were isolated and purified (details below), and then transformed as described above.

mono-Z-Gly derivative **(n=3, X=O, R=COOMe)**. Purified by flash chromatography (n-hexane-ethyl acetate 55:45). Yield: 50%. ^{1}H NMR ($CDCl_3$) δ: 1.57 (1H, t, J=7.83Hz), 1.89-2.05 (4H, m), 2.58 (2H, dt, J=7.83,6.50Hz), 3.09 (2H, t, J=6.50Hz), 3.52-3.60 (8H, m), 3.90 (3H, s), 4.01-4.09 (6H, m), 5.14 (2H, s), 5.41-5.52 (1H, m), 6.55 (2H, dd, J=7.95Hz), 7.23 (1H, t,J=7.95Hz), 7.37 (5H, bs).

Dithiolester **4 (n=3, X=O)**. Purified by flash chromatography (n-hexane-ethyl acetate 53:47). Yield: 90%. ^{1}H NMR ($CDCl_3$) δ: 1.44 (9H, s), 1.93-2.04 (4H, m), 3.01-3.12 (4H, m), 3.47-3.53 (8H, m), 3.90 (3H, s), 3.99-4.14 (6H m), 4.35-4.46 (1H, m), 5.18 (2H, s), 5.51-5.65 (1H, m), 6.51 (2H, dd, J=7.95Hz), 7.21 (1H, t, J=7.95Hz), 7.37 (5H, bs).

mono-Z-Gly derivative **(n=3, X=CH_2, R=COOtBu)**. Purified by flash chromatography (n-hexane-ethyl acetate 7:3). Yield: 49%. ^{1}H NMR ($CDCl_3$) δ: 1.38-1.81 (17H, m), 1.59 (9H, s), 2.54 (2H, dt, J=7.22,6.50Hz), 2.92 (2H, t; J=6.50Hz), 3.97 (4H, t, J=6.50Hz); 4.12 (2H, d, J=6.67Hz), 5.18 (2H, s), 5.79-5.92 (1H, m), 6.53 (2H, d, J=7.15Hz), 7.18 (1H,t, J=7.15Hz), 7.37 (5H, bs).

Dithiolester **3 (n=3, X=CH_2)**. Purified by flash chromatography (n-hexane-ethyl acetate 65:35). Yield: 91%. ^{1}H NMR ($CDCl_3$) δ: 1.38-1.81 (16H, m), 1.46 (9H, s), 1.59 (9H, s), 2.88 (2H, t, J=7.52Hz), 2.91 (2H, t, J=7.22Hz), 3.97 (4H, t, J=6.50Hz), 4.12 (2H,d, J=6.62Hz), 4.29-4.46 (1H, m), 4.93-5.04 (1H, m), 5.18 (2H, s), 5.29-5.40 (1H, m), 6.53(2H,d, J=7.18Hz), 7.18 (1H, t, J=7.18Hz), 7.37 (5H, bs).

mono-Z-Gly derivative **(n=3, X=CH_2, R=COOMe)**. Purified by flash chromatography (n-hexane-ethyl acetate 65:35). Yield: 50%. ^{1}H NMR ($CDCl_3$) δ: 1.22-1.86 (19H, m), 2.56 (2H, dt, J=6.67,6.50Hz), 2.97 (2H, t, J=6.50Hz), 3.90 (3H, s), 3.97 (4H, t, J=6.50), 4.13 (2H, d, J=6.60Hz), 5.63 (2H, s), 5.28-5.42 (1H, m), 6.57 (2H, d, J=7.77Hz), 7.22 (1H, t, J=7.77Hz), 7.37 (5H, bs).

Dithiolester **4 (n=3, X=CH_2)**. Purified by flash chromatography (n-hexane-ethyl acetate 6:4). Yield: 92%. ^{1}H NMR ($CDCl_3$) δ: 1.22-1.86 (19H, m), 1.41 (9H, s), 2.81-2.97 (4H, m), 3.90 (3H, s), 3.97 (4H, t, J=6.50), 4.13 (2H, d, J=6.60Hz), 4.30-4.52 (1H, m), 4.91-5.09 (1H, m), 5.18 (2H, s), 5.24-5.532 (1H, m), 6.51 (2H, d, J=7.77Hz), 7.22 (1H, t, J=7.77Hz), 7.37 (5H, bs).

mono-Z-Gly derivative **(n=2, X=O, R=COOtBu)**. Purified by flash chromatography (n-hexane-ethyl acetate 1:1). Yield: 51%. ^{1}H NMR ($CDCl_3$) δ: 1.59 (9H, s), 2.68 (2H, dt, J=6.50,6.78Hz), 3.12 (2H, t, J=6.50), 3.66 (2H, t, J=6.50Hz), 3.78 (2H, t, 6.10Hz), 4.08-4.21 (6H, m), 5.18 (2H, s), 5.39-5.51 (1H, m), 6.56 (2H, d, J=7.98Hz),

7.22 (1H, t, J=7.98Hz), 7.37 (5H, bs).

Dithiolester **3 (n=2, X=O)**. Purified by flash chromatography (n-hexane-ethyl acetate 1:1). Yield: 89%. ^{1}H NMR ($CDCl_3$) δ: 1.38 (3H, d, J=6.73Hz), 1.48 (9H, s), 1.59 (9H, s), 3.06-3.18 (4H, m), 3.60-3.70 (4H, m), 3.75-3.82 (4H, m), 4.09-4.18 (6H, m), 4.22-4.49 (1H, m), 4.97-5.09(1H, m), 5.18 (2H, s), 5.38-5.48 (1H, m), 6.56 (2H, d, J=7.98Hz), 7.22 (1H, t, J=7.98Hz), 7.37 (5H, bs).

General procedure for the Intramolecular Aminolysis to give **5**.

Compound **3** (0.05 mmol) was dissolved in dry methylene chloride (500 µl) and this solution was then treated with trifluoroacetic acid (TFA) (500 µl) at 0°C under stirring. After 1 hr at 0°C the solvent was evaporated at room temperature. The resulting crude product was dissolved in dry benzene (50 ml) and treated with triethylamine (0.075 mmol). The reaction was stirred at 25°C and monitored by TLC [TLC-absorbance scanner (Camag), λ=254 nm, with correction for the different ϵ_{254} values]. Compound **5** was purified by flash chromatography as indicated below.

Dipeptide derivative **5 (n=3, X=O)**. Purified by flash chromatography (methylene chloride-methanol 96:4). Yield: 80%. ^{1}H NMR ($CDCl_3$) δ: 1.38 (3H, d, J=6.75Hz), 1.52 (1H, t, J=7.05Hz), 1.88-2.02 (4H, m), 2.52 (2H, dt, J=7.05,6.67Hz), 2.99-3.11 (2H, m), 3.48-3.61 (8H, m), 4.00-4.14 (6H, m), 4.62 (1H, dq, J=6.75,6.50Hz), 5.13 (2H, s), 5.88-5.96 (1H, m), 6.51-6.58 (2H, m), 7.19-7.25 (1H, m), 7.32 (5H, bs).

Dipeptide derivative **5 (n=3, X=CH_2)**. Purified by flash chromatography (benzene-ethyl acetate 65:35). Yield 79%. ^{1}H NMR ($CDCl_3$) δ: 1.06-1.72 (20H, m), 2.42 (2H, dd, J=6.50,7.12Hz), 2.72-2.91 (2H, m), 3.77-4.15 (6H, m), 4.70 (1H, dq, J=6.45,6.80Hz), 5.11 (2H, s), 5.92-6.08 (1H, m), 6.49-6.58 (2H, m), 6.90-7.03 (1H, m), 7.21-7.30 (1H, m), 7.32 (5H, bs).

Dipeptide derivative **5 (n=2, X=O)**. Purified by flash chromatography (methylene chloride-methanol 97:3). Yield 78%. ^{1}H NMR ($CDCl_3$) δ: 1.45 (3H, d, J=6.78Hz), 1.59 (1H, t, J=7.07Hz), 2.61 (2H, dt, J=7.07,6.67Hz), 2.92-3.12 (2H, m), 3.55-3.62 (4H, m), 3.71-3.79 (4H, m), 3.90-4.16 (6H, m), 4.54-4.69 (1H, m), 5.13 (2H, s), 6.09-6.23 (1H, m), 6.48-6.53 (2H, m), 7.15-7.26 (1H, m), 7.37 (5H, bs), 7.50-7.74 (1H, m).

General procedure for the Intramolecular Aminolysis to give **6**.

Compound **4** (0.05 mmol) was dissolved in methylene chloride (500 µl) and this solution was then treated with TFA (500 µl) at 0°C under stirring. After 1 hr at 0°C the solvent was evaporated at room temperature. The resulting crude product was dissolved in dry benzene (50 ml) and treated with pivalic acid (5 mmol) and triethylamine (5 mmol), or 2-Pyridone (5 mmol). The reaction was stirred at 25°C and monitored by TLC [TLC-absorbance scanner (Camag), λ=254 nm, with correction for the different ϵ_{254} values]. Compound **6** was purified by flash chromatography as indicated below.

Dipeptide derivative **6 (n=3, X=O)**. Purified by flash chromatography (benzene-ethyl acetate 1:1). Yield 80%. ^{1}H NMR ($CDCl_3$) δ: 1.27-1.43 (3H, m), 1.96-2.03 (4H, m), 2.87 (2H, t, J=6.25Hz), 3.04-3.13 (2H, m), 3.48-3.69 (8H, m), 3.88 (2H, d, J=5.70), 3.90 (3H, s), 4.02-4.14 (4H, m), 4.69 (1H, dq, J=6.60,7.20Hz), 5.12 (2H, s), 5.42-5.59 (1H, m), 6.57 (2H, d, J=7.77Hz), 7.22 (1H, t, J=7.77Hz), 7.37 5H, bs).

Dipeptide derivative **6 (n=3, X=CH_2)**. Purified by flash chromatography (benzene-ethyl acetate 7:3). Yield 75%. ^{1}H NMR ($CDCl_3$) δ: 1.22-1.82 (10H, m), 2.54 (2H, dt, J=6.67,6.90Hz), 2.90 (2H, t, J=6.50Hz), 3.87-4.02 (9H, m), 4.72 (1H, dq, J=6.67,7.20Hz), 5.18 (2H, s), 5.85-5.99 (1H, m), 6.53 (2H, d, J=7.77Hz), 7.22 (1H, t, J=7.77Hz), 7.37 (5H, bs).

Dithiolester **7 (n=3, X=O)**. Triethylamine (40 µl, 0.287 mmol) and DPPA (62 µl, 0.287 mmol) were added to a stirred solution of Ala-Boc (54 mg, 0.287 mmol) in dry DMF (280 µl) at 0°C. After 10 min at 0°C this mixture was treated with a solution of dipeptide derivative **5 (n=3, X=O)** in dry DMF (450 µl) and stirred at 0°C for 2 hr. The reaction mixture was then diluted with ethyl acetate (20 ml) and the organic phase was washed with 5% aq. citric acid (2x2 ml) and brine (1 ml); the organic extracts were dried and evaporated. The crude product was purified by flash chromatography (methylene chloride-methanol 94:6) to give compound **7** in 85% yield. ^{1}H NMR ($CDCl_3$) δ: 1.32 (3H, d, J=7.12Hz), 1.45 (9H, s), 1.58-2.02 (4H, m), 2.95-3.11 (4H, m), 3.45-3.65 (8H, m), 3.86-3.94 (2H, m), 4.08 (4H, t, J=6.50), 4.25-4.42 (1H, m), 4.62 (1H, dq, J=6.75,6.50Hz), 5.85-5.98 (1H, m), 6.54 (2H, d, J=7.77Hz), 7.22 (1H, t, J=7.77Hz), 7.33 (5H, bs).

Tripeptide derivative **8 (n=3, X=O)**. The intramolecular aminolysis to give compound **8** was performed starting from **7** and following the general procedure described above for the synthesis of dipeptides **5** (CH_2Cl_2-TFA, 0°C, 1 hr; evaporation of solvent; benzene-Et_3N). The crude product was purified by flash chromatography (methylene chloride-methanol 9:1) to give tripeptide **8** in 35% yield. ^{1}H NMR ($CDCl_3$) δ: 1.20-1.52 (7H, m), 1.58-2.05 (4H, m), 2.53 (2H, dt, J=6.75,6.67Hz), 2.97-3.09 (2H, m), 3.49-3.68 (8H, m), 3.88-3.97 (2H, m), 4.01-4.16 (4H, m), 4.50-4.73 (2H, m), 5.12 (2H, s), 5.81- 5.99 (1H, m), 6.40-6.49 (1H, m), 6.50-6.60 (2H, m), 7.22 (1H, J=7.75Hz), 7.32 (5H, bs), 7.55-7.71 (1H, m).

Notes and References

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