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An Optically Active Nucleophile That Catches Its Substrate By Two Points

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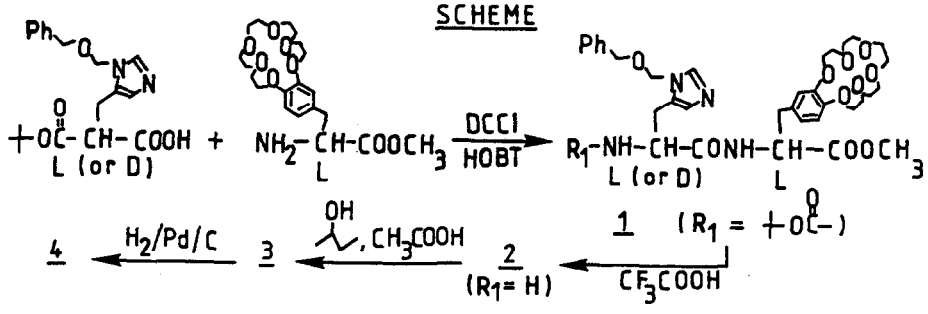
Abstract: Two diastereoisomeric cyclic dipeptides are obtained by coupling L or D-His with L-(crowned DOPA). The kinetic data on the reaction of p-nitrophenyl esters of protonated amino-acids and peptides with these His derivatives are reported.

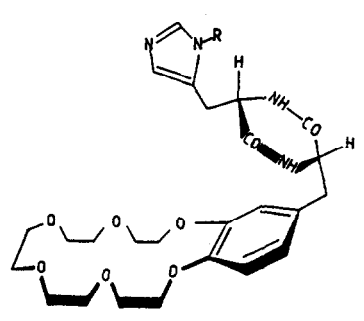
The *de novo* construction of molecular receptors often requires a heavy effort of organic synthesis. The affinity sites and, if needed, the catalytic sites have to converge in space. This problem has been brilliantly solved in some instances by using available tripods like the nitrogen atom (cryptands)[1], Kemp's triacid (Rebek chemistry)[2] or chiral crowns with C_2 symmetry (Lehn, Cram)[3]. A three point ligand has to be assembled in order to discriminate a chiral object like an amino-acid from its enantiomer. A cyclic tripeptide including amino-acids with properly engineered side-chains should create such a discriminating pocket with a minimum of synthetic effort. One of the residues would catch the carboxyl function, another the α-amino function and the third one would attract or repulse the side-chain of the substrate. We describe here a first step in this direction: the synthesis of a cyclic dipeptide able to catch an amino-acid p-nitrophenyl ester by two points. The compound was obtained by coupling a nucleophilic catalyst His with an α-ammonium complexing function, crowned DOPA.

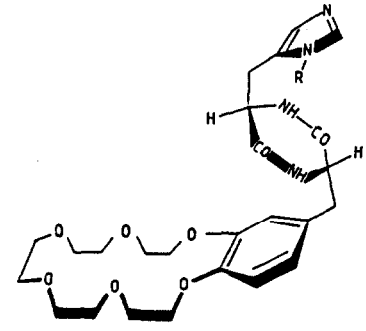
Synthesis of the linear fully protected dipeptides L(orD)-His-L-(crowned DOPA) **1LL, 1DL** (SCHEME): 1-hydroxybenzotriazole (0.9 mmole) and dicyclohexylcarbodiimide (1 mmole) were added to a solution of N(α)-t-butoxycarbonyl-N(π)-benzyloxymethyl-L-histidine (0.84 mmole) in dry DMF (5 cc) at 0°c. The solution was stirred at 0°c for one hour, then at R.T. for one hour. L-[3,4-(1,4,7,10,13,16-hexaoxahexadecamethylene) phenyl] alanine methyl ester[4] (0.76 mmole) dissolved in DMF (6 cc) was added. The mixture was stirred overnight, filtered and evaporated. The residue was partitioned between $NaHCO_3$ (10%) and ethyl acetate. The organic phase was extracted with citric acid (10%). The citric acid phase was neutralized with solid $NaHCO_3$ and extracted with ethyl acetate. This last extract was dried ($MgSO_4$) and evaporated to give the title product as a white foam (0.54 mmole, 71%). **1LL** $[\alpha]_D^{20} = +11$ (c=0.55, CH_2Cl_2). **1DL** $[\alpha]_D^{20} = +9$ (c=0.43, CH_2Cl_2)[5].

Synthesis of the cyclic dipeptides cyclo-L(or D)-His-L-(crowned DOPA) **4LL, 4DL**: **1LL** (0.8 mmole) was dissolved in trifluoroacetic acid (3 cc). The acid was evaporated after 0.5h. The residue was partitioned between $NaHCO_3$ and CH_2Cl_2. The dried extract ($MgSO_4$) was evaporated to give **2LL** that was dissolved in a 0.1 M solution of acetic acid in 2-butanol (11.5 cc) and refluxed for three hours[6]. The solvent

SCHEME





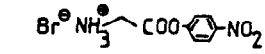
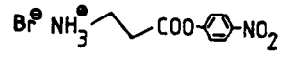
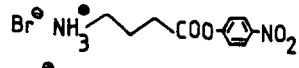
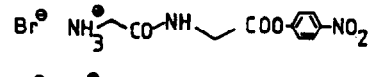
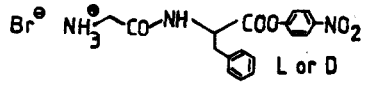


3LL R = Ph-O- 4LL R = H

3DL R = Ph-O- 4DL R = H

TABLE

Pseudo first order rate constants ($s^{-1} \times 10^5$, 25°c) for the release of p-nitrophenolate ions in the buffer (k_{spont}) or in the presence of an excess of 4DL (4LL, resp.).

SUBSTRATE		k_{spont}	k_{4DL}	k_{4LL}
	a	0.84	28.4	40.5
	b	1.5	46.5	256
	c	4600	2100	4700
	d	13.5	12.0	57.5
 L or D	e	18.8	20.7	134 (L) 124 (D)

was evaporated. The residue was partitioned between $NaHCO_3$ (10%) and CH_2Cl_2. The dried extract ($MgSO_4$) was evaporated and the residue chromatographed on neutral alumina [CH_2Cl_2/CH_3OH (2-10%)] to give **3LL** (yield 57%[5]; **3DL**, 41%). **3LL** (0.15 mmole) was dissolved in 80% aqueous acetic acid (15 cc). A catalyst was added (Pd/C 10%, 10 mg) and the mixture was hydrogenated in a Parr apparatus (30 psi, 8 h). The solution was filtered through celite, evaporated and coevaporated with water and finally ethanol. The deprotection yield was quantitative. **4LL** $[\alpha]_D^{20}$= -29 (c=0.64, C_2H_5OH). **4DL** $[\alpha]_D^{20}$= +6 (c=1, C_2H_5OH).

The rates of release of the p-nitrophenolate ion from various protonated amino-acids and peptides p-nitrophenyl esters, in the absence or in the presence of compounds **4LL** or **4DL**, were compared (TABLE)[7]. The nucleophilic and complexing centers of **4LL** may converge in space[8], whereas they diverge in most conformations of **4DL**[9]. A surprise was the inhibition of the solvolysis of substrates (**c**) and (**d**) by **4DL**. The same effect was observed when other crowns were added to these substrates [substrate (**c**) + dicyclohexyl-18C6, k=800 10^{-5} s^{-1}; + benzo-18C6, k=1700 10^{-5} s^{-1}; substrate (**d**) + benzo-18C6, k=7.8 10^{-5} s^{-1}]. Substrate (**c**) cyclizes to give a five membered lactame and substrates (**d**) and (**e**), to give diketopiperazines. The starting linear molecules have to be deprotonated in order to cyclize. Complexation of the ammonium form by a crown ether lowers the concentration of the free amino form and slows down the cyclization. This fortuitous observation could throw the basis of a general method to compare the affinities of various crowns for a primary ammonium salt like (**c**). The higher the affinity, the slower the cyclization.

Substrates (**c**)-(**e**) were however not suitable for our first aim, i.e., for comparing the rates of N-acylation of **4DL** and **4LL** by electrophilic esters. The results with two other substrates (**b**) and, to a lesser extend, (**a**) show that the more crowded **4LL** is a better nucleophile than **4DL**. This is unusual, as cyclo(L-Phe-L-His) and cyclo(L-Tyr-L-His) are less reactive towards p-nitrophenylacetate than cyclo(D-Phe-L-His) and cyclo(D-Tyr-L-His), respectively[10, 11]. It is thus most likely that compound **4LL** catches (**a**) and (**b**) by two points: the primary ammonium function is complexed by the crown, facilitating the attack of the carboxyl by an imidazole nitrogen[12]. There is an optimum distance between the ammonium and carboxyl functions: the ratio k_{LL}/k_{DL} is larger for β than for α-amino-acids. The trans geometry of **4DL** does not allow the same cooperative action of both sites. These preliminary kinetic results are now extended to other substrates to judge of a possible chiral recognition. Cyclo[L-Cys-L-(crowned DOPA)] as well as cyclic tripeptides are under study.

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5 The ^1H and ^{13}C NMR spectra of all the described compounds were fully consistent with the structures. C, H, N Analysis for **3LL**: calc. for $C_{33}H_{42}N_4O_9$, C, 62.06; H, 6.63; N, 8.77. Found, C, 62.20; H, 6.69; N, 8.54.

6 Classical conditions for diketopiperazine formation. Suzuki, K., Sasaki, Y., Endo, N. and Mihara, Y., Chem. Pharm. Bull. **29**, 233 (1981).

7 We worked in CH_2Cl_2/C_2H_5OH (95:5). This solvent was buffered with a mixture of N-ethylmorpholine (0.03 M) and trifluoroacetic acid, in proportion calculated to give pH 7 in water (ref. 13). The pKa of the His resisue is about 6 (ref. 11).The p-nitrophenyl esters were 5 10^{-5} M. The nucleophile **4DL** (resp. **4LL**) was 0 M (k_{spont}) or 4 10^{-4} M (k_{4DL}, k_{4LL}). The spectrum of the mixture was monitored at 315 nm.

8 For example, cyclo(L-His-L-His) form a Cu(II) complex where both side-chain coordinate the cation (Kojima, Y., Chem. Lett., **1981**, 61). N-Acetyl-cyclo(L-His-L-Tyr) rapidly transfer intramolecularly the acetyl group from the histidyl to the tyrosyl residue (ref. 11). An aromatic residue of cis-diketopiperazines is folded back over the piperazine ring [Kopple, K. D. and Ohnishi, M., J. Am. Chem. Soc. **91**, 962 (1969). Kojima, Y., Yamashita, T., Nishide, S., Hirotsu, K. and Higuchi, T., Bull. Chem. Soc. Jpn. **58**, 409 (1985). Gdaniec, M. and Liberer, B., Acta Cryst. **C42**, 1343 (1986)].

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