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Serine-containing 10-membered cyclodepsipeptides

Synthesis and molecular structure of $PhCH_2CO$-DSer-Pro-Pro- and $PhCH_2CO$-DSer-Pro-DPro-

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As a part of a research program aimed at studying synthesis and conformation of small ring peptides, the cyclization of diastereoisomeric *N*-phenylacetyl-seryl-propyl-proline tripeptides has been examined. Two 10-membered peptide lactones, $PhCH_2CO$-DSer-Pro-Pro- **5a** and $PhCH_2CO$-DSer-Pro-DPro- **5b**, have been isolated by treating the corresponding linear *p*-nitrophenyl esters with DBU in dry benzene. In these two compounds the serine lactone fragment (a common structural feature of several bioactive cyclodepsipeptides) is inserted into a highly strained small ring system. The conformation in the crystal of **5a** and **5b** has been studied by X-ray analysis. Both the 10-membered rings of **5a** and **5b** adopt an overall *cis-cis-trans* conformation in which the lactone junction is *trans*. The deviations from planarity of the peptide units vary from $\Delta\omega = 30°$ for the DSer—Pro bond in **5b** to $\Delta\omega = 5$–$6°$ for the DSer—Pro bond in **5a** and Pro—DPro bond in **5b**. The skeletal atoms of **5b**, containing the Pro-DPro sequence, are related by a pseudo-symmetry mirror plane passing through the Pro carbonyl and the opposite DSer $C^{\beta}H_2$ group. In both the molecules the exocyclic amide bond adopts an extended conformation with respect to the DSer-Pro ring junction; this arrangement gives rise to a C_5-type ring structure which is well evidenced in the case of **5a**.

Key words: conformation; cyclodepsipeptides; peptide lactones; proline; serine; tripeptides; X-ray structure

The present report is a part of our continuing interest in the chemistry and conformation of cyclotripeptides and cyclotridepsipeptides (tripeptide lactones) (1–6). These compounds represent, after dioxopiperazines and dioxomorpholines, the smallest cyclopeptide systems and are characterized by unusual chemical and structural properties, basically connected with the conformational constraint of the nine-membered ring, deviation from planarity of the peptide bonds and short transannular distances leading to isomerizations and rearrangements. In this field we described previously synthesis and conformational preferences of a group of 10-membered cyclotripeptides and cyclotridepsipeptides possessing the general structure -βXaa-Yaa-Pro-, where βXaa represents a β-amino- or a β-hydroxy-acid residue (1, 2, 4–6). These systems are characterized by low conformational heterogeneity, and present, as compared with the well known nine-membered cyclotripeptides, a less pronounced propensity towards transannular interactions and molecular rearrangements. This feature allowed the design of valuable models for detailed conformational investigations (6).

As a continuation of these studies we have examined

Abbreviations follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature as given in *Eur. J. Biochem.* (1984) **138**, 9–37. Additional abbreviations: AcOH, acetic acid; DBU, 1,8-diazabicyclo[5,4,0]undec-7-ene; DCCI, dicyclohexylcarbodiimide; DMSO, dimethyl sulfoxide; EtOAc, ethyl acetate; HOBt, 1-hydroxybenzotriazole; Hyb, *S*-3-hydroxybutyric acid; MeAnt, *N*-methylanthranilic acid; MeOH, methanol; NNM, *N*-methylmorpholine; THF, tetrahydrofuran; TLC, thin-layer chromatography; TMS, tetramethyl silane.

the synthesis and conformation of ten-membered tripeptide lactones of the R-CO-Ser-Pro-Pro- type, containing two proline residues in addition to a serine unit as the β-hydroxyacidic component. As previously pointed out by Mauger & Stuart (7), the lactone unit in which a serine or threonine side-chain is *O*-acylated by the carboxy terminus of the peptide is a key structure feature of several bioactive natural compounds. In these peptide lactones the exocyclic serine or threonine amino group provides the handle to which a carboxylic acid, usually responsible for the activity, is bound. This is the case of quinoxaline antibiotics (8) and siderophore enterobactin, a trimer lactone of *N*-2,3-dihydroxybenzoyl serine (9). In more strained and complex systems, such as the tyrosinase inhibitor microviridin (10), the serine residue is part of a peptide lactone, bridged through the serine amino group to a larger tricyclic peptide structure.

In the case of the RCO-Ser-Pro-Pro- models reported here, the general lactone fragment has been inserted into a highly strained ring system in order to reduce the conformational variability of the peptide backbone and gain information on the spatial relationship between the backbone elements and the exocyclic carboxyamide moiety. To our knowledge these compounds represent the smallest cyclodepsipeptide models obtained through the "side chain to end" mode of cyclization (11).

RESULTS AND DISCUSSION

At variance with cyclopeptides containing at least one secondary amide bond in the ring, the present models cannot be synthesized by using the hydroxyacyl insertion method, a strategy which minimizes the oligomerization reactions frequently encountered during the synthesis of small ring cyclopeptides. Thus, the end-to-end cyclization through ester bond formation between the free serine β-hydroxyl and the activated carboxylic group of the *C*-terminal proline was examined.

In a first approach the homochiral *N*-phenylacetyl tripeptide $PhCH_2CO$-Ser-Pro-Pro-ONp was selected as suitable linear precursor. Adoption of different cyclization conditions led, however, to unsatisfactory results owing to the formation of cyclo-oligomers, among which the 40-membered tetrameric peptide lactone was the prevailing species (12). The two heterochiral isomers Ph-CH_2-CO-DSer-Pro-Pro-ONp and Ph-CH_2-CO-DSer-Pro-DPro-ONp, **4a** and **4b**, respectively, corresponding to the DLL and DLD sequences, were then synthesized and subjected to cyclization (Fig. 1). In contrast to the results obtained with the homochiral precursor, both **4a** and **4b** gave, by treatment with DBU in dry benzene (3), good yields of the corresponding monomeric lactones $PhCH_2CO$-DSer-Pro-Pro- **5a** and $PhCH_2CO$-DSer-Pro-DPro- **5b**. Note that, since the serine side-chain is involved in the ring system instead of the serine α-amino group, the $PhCH_2CO$-DSer-Pro-Pro- heterochiral stereoisomer **5a** presents all three α-hydrogen atoms on the same side of the mean plane of the cyclopeptide ring, a stereochemical feature typical of homochiral cyclopeptides. Formation of the isomeric *O*-phenylacetyl homodetic nine-membered cyclotripeptide resulting from an N to O acyl shift, a frequently encountered side-reaction at the Ser and Thr residues (13), is not observed. In accordance with the assigned lactone structure, treatment of **5a,b** with hydrazine hydrate gives only the corresponding *N*-phenylacetyl tripeptide hydrazides; no traces of $PhCH_2CO$-$NHNH_2$ are evidenced.

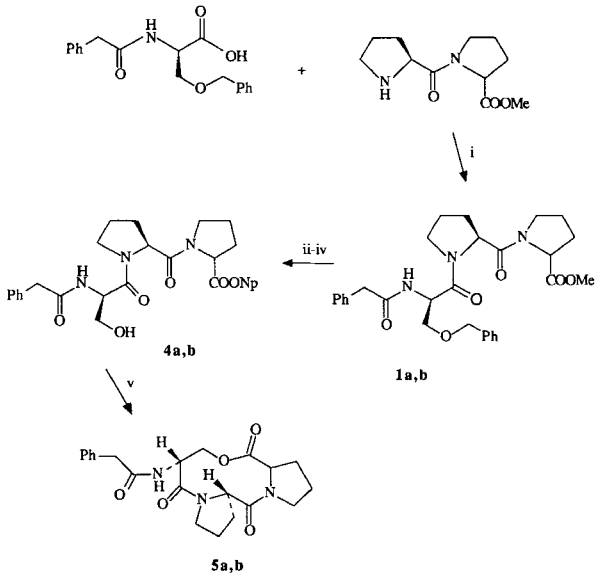

FIGURE 1

Synthesis of cyclotridepsipeptides **5a** and **5b**. Compounds **a** and **b** contain the sequence Pro-Pro and Pro-DPro, respectively. Reagents: (i) DCCl, HOBt; (ii) 2 N NaOH; (iii) H_2, 5% Pd/C; (iv) DCCl, *p*-nitrophenol; (v) DBU.

Crystals of compounds **5a** and **5b** suitable for crystal structure determination were grown from EtOAc solutions by slow evaporation. A perspective view of the molecular structures of **5a** and **5b** is reported in Figs. 2 and 3; the fractional atomic coordinates of both compounds are reported in Table 1. Bond lengths and bond angles (Table 2) are in general agreement with the previously reported values for proline-containing small ring cyclopeptides (1–6, 14). Attention should be drawn to the high value of the ring angles at N_2 in **5a** and N_3 in **5b**, both corresponding to *cis*-planar peptide nitrogen atoms. Deviations from the standard values are also shown by the ring angles at the tetrahedral carbon atom C_2^α in **5b** (τ_2), at the sp^2 carbon atoms C'_2 and C'_3 in **5b** and at C'_3 in **5a**. A significant compression of the tetrahedral angle is observed in **5a** at the extra-ring angle of DSer C_1^α atom (τ_1; Table 2). Interatomic distances across the ring which are significantly shorter

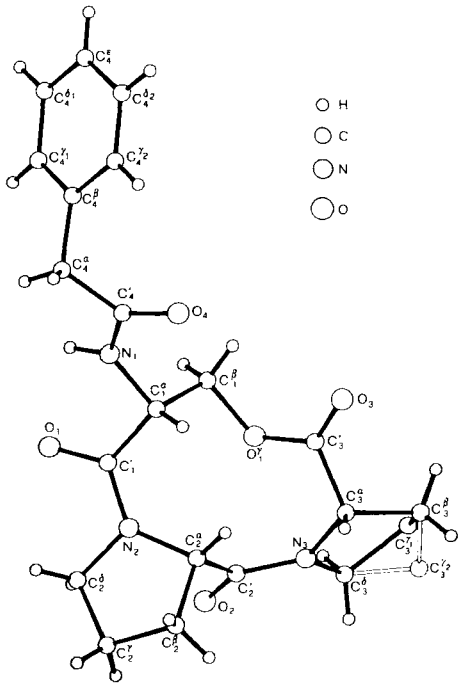

FIGURE 2

A perspective view of the molecular structure of Ph-CH_2-CO-DSer-Pro-Pro- **5a**. The outlined bonds concern disordered proline C_3^{γ} atoms.

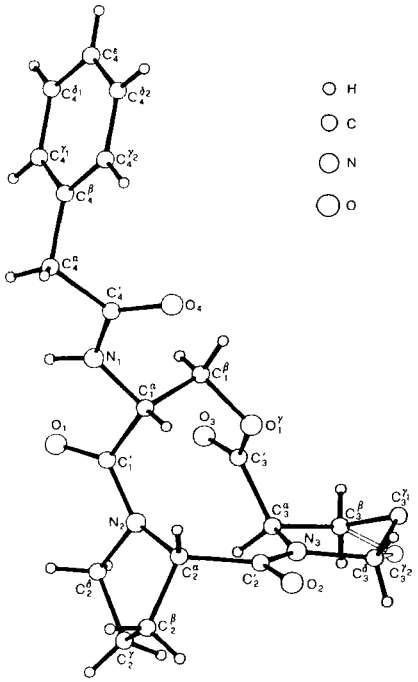

FIGURE 3

A perspective view of the molecular structure of Ph-CH_2-CO-DSer-Pro-DPro- **5b**. The outlined bonds concern disordered proline C_3^{γ} atoms.

than the sum of the van der Waals radii involve, in both the molecules, the atoms of the lactone unit (O_1^{γ} in **5a** and C_3^{γ} in **5b**); a short contact is also observed in **5b** between the two α-hydrogen atoms of the fragment DSer-Pro Figs. 3 and 4 and Table 3).

The backbone conformation of **5a** and **5b** is described by the sequence of torsion angles reported in Table 4. The conformation of the exocyclic amide bond is *trans* in both **5a** and **5b**, with a significant distortion from planarity in the case of **5a** ($\omega_4 = 168.0°$). Each ring is characterized by the presence of two *cis*-configurated peptide bonds (ω_1 and ω_2) and by a *trans* lactone unit (ω_3). Thus, the overall backbone conformation of the two rings can be described as *cis-cis-trans*. An analogous arrangement of the three CO—X (X = N; O) ring junctions has been found in all the ten-membered homodetic and heterodetic cyclotripeptides studied so far, with the notable exception of cyclo(-MeAnt-Phe-Pro-) (4), which adopts an all-*cis* backbone conformation analogous to the symmetrical crown conformation adopted by the homochiral nine-membered cyclotripeptides (14).

The conformational constraint of ten-membered ring induces different distortion on the three ring junctions. Thus, in both **5a** and **5b** (Table 4) one of the two amide bonds is practically planar ($\Delta\omega \approx 5°$), whereas the other

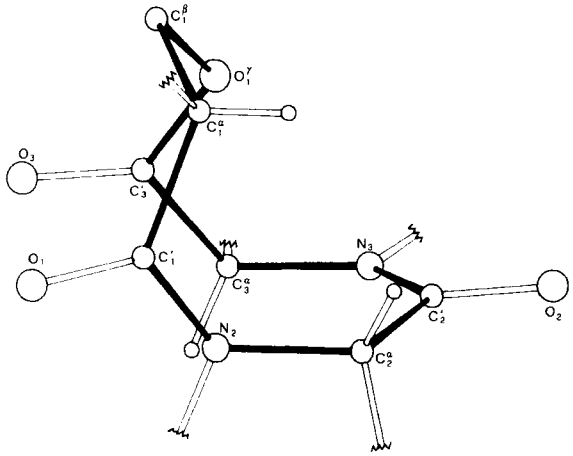

FIGURE 4

Side view of the cyclic backbone of Ph-CH_2-CO-DSer-Pro-DPro- **5b**, showing the pseudo-symmetry mirror plane. Backbone bonds are printed in bold-face.

deviates significantly from planarity; in particular, the DSer—Pro bond of **5b** ($\Delta\omega_1 = 30.1°$) presents one of the largest deviations observed so far. The CO—O lactone group adopts, in both models, a significantly distorted *trans*-conformation [$\Delta\omega_3 = 21.4$ (**5a**) and 25.1° (**5b**)],

TABLE 1.

Fractional atomic coordinates (with e.s.d.s in parentheses) and thermal parameters

Atom	*x*	*y*	*z*	*B*(eq)[a]
O_1^{γ}	0.7194(6)	0.1623(3)	0.4303(1)	3.9(1)
C_1^{β}	0.605(1)	0.1026(5)	0.4693(2)	4.3(1)
C_1^{α}	0.589(1)	−0.0151(4)	0.4523(2)	3.9(1)
N_1	0.479(1)	−0.0788(4)	0.4906(2)	5.2(1)
C_1'	0.444(1)	−0.0302(4)	0.4062(2)	3.7(1)
O_1	0.2463(7)	−0.0569(3)	0.4109(1)	4.6(1)
N_2	0.5333(7)	−0.0169(3)	0.3610(1)	3.4(1)
C_2^{α}	0.7565(9)	0.0205(4)	0.3458(2)	3.3(1)
C_2^{β}	0.805(1)	−0.0511(5)	0.3007(2)	4.1(1)
C_2^{γ}	0.577(1)	−0.0729(5)	0.2775(2)	4.8(2)
C_2^{δ}	0.402(1)	−0.0520(5)	0.3177(2)	4.4(1)
C_2'	0.738(1)	0.1402(4)	0.3291(2)	3.3(1)
O_2	0.5886(7)	0.1665(3)	0.3009(1)	4.6(1)
N_3	0.8973(9)	0.2107(3)	0.3441(2)	4.0(1)
C_3^{α}	1.054(1)	0.1979(4)	0.3854(2)	4.0(1)
C_3^{β}	1.184(1)	0.3054(6)	0.3869(3)	6.8(2)
$C_3^{\gamma 1}$	1.026(3)	0.384(1)	0.3674(5)	5.9(4)[b]
$C_3^{\gamma 2}$	1.113(3)	0.366(1)	0.3396(6)	5.3(5)[b]
C_3^{δ}	0.886(1)	0.3247(4)	0.3271(2)	5.2(2)
C_3'	0.942(1)	0.1735(4)	0.4348(2)	3.8(1)
O_3	1.0472(8)	0.1617(4)	0.4715(1)	5.6(1)
C_4'	0.584(1)	−0.1280(5)	0.5274(2)	4.8(2)
O_4	0.784(1)	−0.1144(5)	0.5346(2)	6.9(2)
C_4^{α}	0.445(2)	−0.2073(5)	0.5565(2)	6.0(2)
C_4^{β}	0.413(1)	−0.1767(4)	0.6101(2)	4.1(1)
$C_4^{\gamma 1}$	0.585(1)	−0.1899(4)	0.6438(2)	4.0(1)
$C_4^{\gamma 2}$	0.210(1)	−0.1410(7)	0.6266(3)	7.0(2)
$C_4^{\delta 1}$	0.554(1)	−0.1691(5)	0.6932(2)	5.3(2)
$C_4^{\delta 2}$	0.178(2)	−0.1220(8)	0.6772(4)	9.2(3)
C_4^{ε}	0.347(2)	−0.1361(7)	0.7092(3)	6.9(2)
Cyclotridepsipeptide **5b**				
O_1^{γ}	0.2979(5)	0.7260	0.2379(4)	4.2(1)
C_1^{β}	0.2275(7)	0.6849(9)	0.3358(6)	4.4(2)
C_1^{α}	0.3407(6)	0.6867(8)	0.4453(5)	3.6(1)
N_1	0.2750(6)	0.6487(7)	0.5463(5)	3.8(1)
C_1'	0.4625(6)	0.5985(7)	0.4258(5)	3.3(1)
O_1	0.4514(6)	0.4930(6)	0.4522(4)	4.4(1)
N_2	0.5734(5)	0.6389(7)	0.3694(4)	3.2(1)
C_2^{α}	0.6302(7)	0.7630(7)	0.3701(5)	3.4(1)
C_2^{β}	0.7955(8)	0.7444(9)	0.3919(7)	5.2(2)
C_2^{γ}	0.8173(9)	0.625(1)	0.3295(9)	6.3(3)
C_2^{δ}	0.6875(7)	0.5493(9)	0.3520(6)	4.3(2)
C_2'	0.5938(6)	0.8468(7)	0.2632(5)	3.5(1)
O_2	0.6288(6)	0.9524(6)	0.2793(5)	4.8(1)
N_3	0.5343(6)	0.8104(7)	0.1559(4)	3.9(1)
C_3^{α}	0.4804(8)	0.6919(7)	0.1147(5)	4.0(2)
C_3^{β}	0.430(1)	0.714(1)	−0.0148(6)	7.0(3)
$C_3^{\gamma 1}$	0.386(2)	0.840(1)	−0.027(1)	4.6(4)
$C_3^{\gamma 2}$	0.464(3)	0.827(2)	−0.048(2)	5.1(6)
C_3^{δ}	0.503(1)	0.9022(9)	0.0632(6)	5.5(2)
C_3'	0.3592(8)	0.6423(8)	0.1764(6)	4.2(2)
O_3	0.3226(7)	0.5372(7)	0.1682(5)	6.1(2)
C_4'	0.1803(7)	0.7196(8)	0.5926(6)	4.1(2)
O_4	0.1360(6)	0.8160(7)	0.5483(5)	5.3(1)

TABLE 1.

(continued)

Atom	*x*	*y*	*z*	*B*(eq)[a]
C_4^{α}	0.1318(7)	0.6749(9)	0.7082(6)	4.7(2)
C_4^{β}	−0.0314(7)	0.6676(8)	0.7025(5)	3.6(1)
$C_4^{\gamma 1}$	−0.105(1)	0.5586(9)	0.6792(8)	5.2(2)
$C_4^{\gamma 2}$	−0.1134(8)	0.7692(8)	0.7196(7)	4.5(2)
$C_4^{\delta 1}$	−0.256(1)	0.552(1)	0.6762(8)	5.8(2)
$C_4^{\delta 2}$	−0.2667(9)	0.760(1)	0.7153(8)	5.5(2)
C^{ε}	−0.3357(8)	0.653(1)	0.6940(7)	5.4(2)
A_1	0.945(3)	0.641(3)	0.016(2)	15.9(7)[b,c]
A_2	0.970(3)	0.777(3)	0.019(2)	14.9(7)[b,c]
A_3	0.997(2)	0.525(2)	−0.003(2)	12.0(5)[b,c]
A_4	0.811(3)	0.585(3)	0.028(2)	17.9(9)[b,c]

[a] $B(\text{eq}) = (4/3)\ \Sigma_{ij}\ a_i \cdot a_j\ b_{ij}$.

[b] This atom was refined isotropically.

[c] Symbol A denotes solvent atoms (see text).

and this can be related to the low rotational barrier of esters as compared with amides.

Together with the distortion of the peptide junctions ($\Delta\omega$), the pyramidality of the nitrogen atoms, as revealed by the displacement d(N) (Å) from the plane of its three substituents, has been determined. All the amide nitrogen atoms are practically flat, except the nitrogen atoms involved in the two distorted amide bonds (N_3 in **5a** and N_2 in **5b**), which are both significantly pyramidal: $d(N_3) = 0.113$ Å in **5a** and $d(N_2) = -0.201$ Å in **5b**. It should be mentioned in this context that, in agreement with the high deviation from planarity ($\Delta\omega_1 = 30.1°$) and the significant nitrogen pyramidalization, the CO—N_2 bond in **5b** exhibits the greatest C′ – N length (1.356 Å; Table 2) (6).

TABLE 2

Selected bond lengths (Å) and ring angles (°) for $PhCH_2CO$-D-*Ser-Pro-Pro-* **5a** *and* $PhCH_2CO$-D*Ser-Pro-DPro-* **5b**, *with e.s.d.s in parentheses*

	5a	**5b**
Bonds		
C_2'—N_2	1.344(7)	1.356(8)
C_2'—N_3	1.347(7)	1.348(9)
C_3'—O_1^{γ}	1.331(7)	1.333(8)
C_4'—N_1	1.323(8)	1.335(9)
Angles		
C_1^{β}—C_1^{α}—C_1'	113.3(4)	108.0(6)
C_1^{α}—C_1'—N_2	120.7(5)	118.4(7)
C_1'—N_2—C_2^{α}	130.6(4)	126.6(6)
N_2—C_2^{α}—C_2' τ_2	108.6(4)	121.0(6)
C_2^{α}—C_2'—N_3	118.6(4)	125.0(7)
C_2'—N_3—C_3^{α}	127.3(4)	130.5(6)
N_3—C_3^{α}—C_3' τ_3	114.6(5)	114.6(6)
C_3^{α}—C_3'—O_1^{γ}	111.8(4)	113.0(7)
C_3'—O_1^{γ}—C_1^{β}	116.6(4)	117.4(5)
O_1^{γ}—C_1^{β}—C_1^{α}	107.2(4)	108.1(5)
N_1—C_1^{α}—C_1' τ_1	105.3(5)	109.1(6)

TABLE 3

Selected intramolecular short distances (Å) for $PhCH_2CO$-D-*Ser-Pro-Pro-* **5a** *and* $PhCH_2CO$-D*Ser-Pro-*D*Pro-* **5b**

	5a	**5b**
$O_1^{\gamma} \cdots O_2^{\alpha}$	2.89	
$O_1^{\gamma} \cdots C_2'$	2.76	
$C_1' \cdots C_3'$		2.95
$N_2 \cdots C_3'$		2.78
$H_1^{\alpha} \cdots H_2^{\alpha}$	2.30	2.08
$H_2 \cdots H_3$	2.27	
$H_1 \cdots O_1$	2.20	2.35
$N_1 \cdots O_1$	2.58	2.69

Both **5a** and **5b** have the pyrrolidine ring of the Pro_2 residue in the envelope (C_s symmetry) conformation which can be described as C_s-C^{α}-endo in **5a** and C_s-C^{β}-exo in **5b** (15). As frequently found in crystal structures of proline-containing peptides, the C^{γ} atoms of Pro_3 residues in both **5a** and **5b** are disordered (16). By taking into account the two positions assigned to the C_3^{γ} atoms in the crystal (Figs. 2 and 3), all the resulting Pro_3 pyrrolidine rings can be described as half-chair (C_2 symmetry) conformations.

Although several medium-sized cyclodepsipeptides have been studied, only few data are at the present available on small ring models. It thus seemed interesting to correlate the backbone conformation of **5a**,**b** with that of the previously studied ten-membered cyclotridepsipeptide -Hyb-Phe-Pro- (**6**) (1), characterized by the presence of only one proline residue in the

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