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Conformationally Constrained Tryptophan Analogs. Synthesis of (±)-(Z)- and (±)-(E)-2-Amino-2,3-methano-3-(indol-3-yl)-propanoic Acids

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Abstract: The syntheses of (±)-(Z)- and (±)-(E)-2-amino-2,3-methano-3-(indol-3-yl)-propanoic acids (**8** and **15**) are reported. Key step in their preparation is the cyclopropanation of an olefinic azlactone derivative, followed by a suitable deprotection sequence.

INTRODUCTION

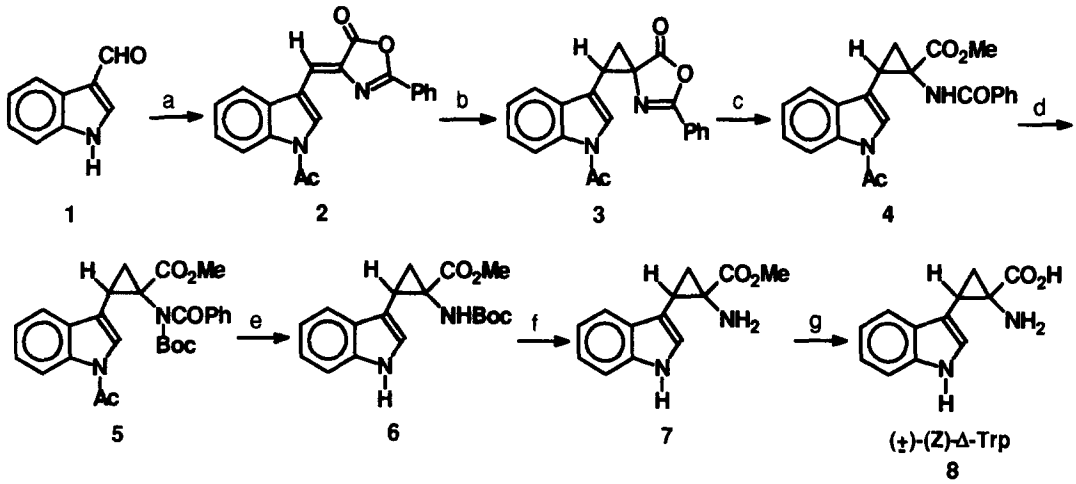
Cyclopropane amino acids (Δ-AA) have attracted a good deal of interest in recent years.[1] Several naturally occurring, biologically active members of this class have been identified and submitted to structural modifications aimed at improving their potency and selectivity. At the same time, the cyclopropyl group is increasingly being introduced in the side chain of proteinogenic amino acids,[2] most frequently in the C2-C3 position, in order to induce conformational restriction with minimal steric interference. In an ongoing program aimed at the synthesis of conformationally restricted tryptophan derivatives,[3] we required (±)-(Z)- and (±)-(E)-2,3-methano-tryptophans [(±)-(Z)- and (±)-(E)-Δ-Trp] **8** and **15**, respectively. Previously, Bernabé et al.[4] have obtained trace amounts of intermediates on route to **8** and **15** starting from a tioazlactone precursor. More recently, Bruncko et al.[5] have described the synthesis of an enantiopure (Z)-Δ-Trp derivative as an intermediate for the preparation of β-substituted tryptophan derivatives. Here we describe the first synthesis of (±)-(Z)- and (±)-(E)-Δ-Trp (**8** and **15**) by the route depicted in Schemes 1 and 2.

CHEMISTRY

The first step in our synthetic scheme has been the reaction of 3-formyl indole (**1**) with hippuric acid in Ac_2O/AcONa at 80 °C for 40 min to give 2-phenyl-4-(N-acetyl-3-indolylmethylene)-oxazol-5-one in 65% yield and 4:1 (Z/E) stereoselectivity.[6]

The more abundant isomer was unequivocally assigned as Z on the basis of NOE measurements performed on the two benzamido-derivatives **4** and **11**. Indeed, a mutual NOE was found between 3-C*H* and N*H* for compound **4**, thus pointing out a Z stereochemistry, while a mutual NOE was found between 3-C*H* and CH_2 for compound **11**, thus indicating, in this case, an E stereochemistry. During the reaction, it was possible to observe the formation of a precipitate that was filtered, identified[7] as the pure (Z)-2-phenyl-4-(N-acetyl-3-indolylmethylene)-oxazol-5-one (**2**, 46%) and treated with an ethereal solution of diazomethane.[8] Flash chromatography (light petroleum-AcOEt, 8:2) of the reaction product and crystallization (light petroleum-AcOEt) afforded the corresponding (±)-(Z)-Δ-Trp derivative **3** (46%) whose structure was confirmed by spectroscopic and analytical data.

Scheme 1



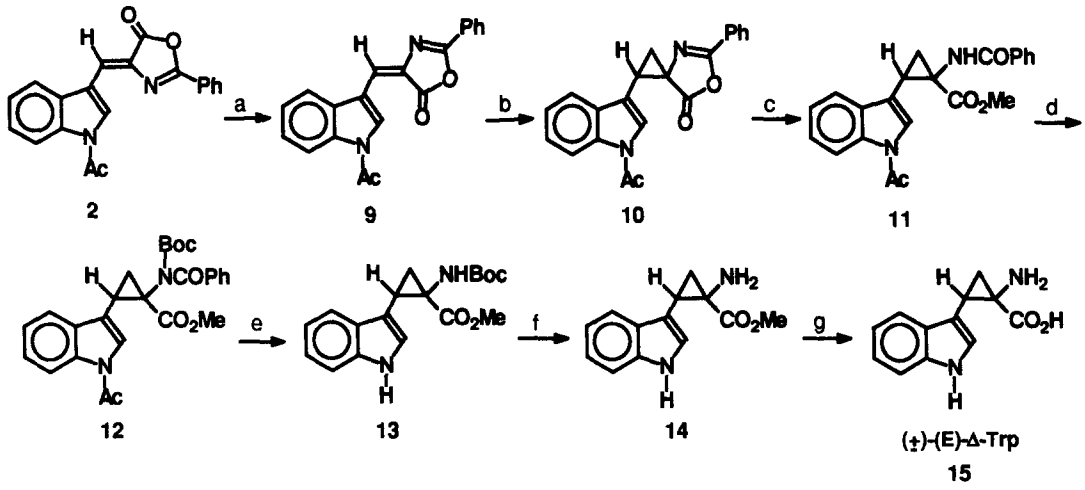
a) Hippuric acid, AcONa, Ac_2O, 80 °C; b) CH_2N_2, ether, rt; c) DMAP, MeOH, rt; d) $(Boc)_2O$, DMAP, CH_2Cl_2, rt; e) $NH_2NH_2 \cdot H_2O$, MeOH, rt; f) HCl, AcOEt, rt; g) i. 1N $LiOH \cdot H_2O$, dioxane, rt; ii. Dowex 50X2-200, 10% Py.

Several attempts to deprotect **3** in a variety of acidic and basic conditions failed and a longer route to the free amino acid **8** was needed. Thus, 4-N,N-dimethylaminopyridine (DMAP)-catalyzed methanolysis[9] of **3** followed by treatment[10] of the N-benzamido-ester **4** thus obtained (85%) with di-*t*-butyl dicarbonate and DMAP afforded the corresponding N-Boc derivative **5** in 95% yield. Hydrazinolysis[11] of **5** in absolute methanol (**6**, 87%) followed by removal[9] of the N-Boc group (12N HCl, AcOEt, rt, 30 min, **7**, 98%), further hydrolysis with LiOH in dioxane-water[12] of ester **7** thus obtained and ion exchange resin chromatography on Dowex 50x2-200 with 10% pyridine afforded (±)-(Z)-Δ-Trp (**8**) in 26% yield, with an overall yield from **1** of 3.8%.

The starting material for the preparation of (±)-(E)-Δ-Trp **15** was the isomeric E-azlactone derivative **9** obtained by treating[6] a suspension of **2** in a 48% HBr solution kept at 0 °C under stirring with a stream

of HBr (Scheme 2). The reaction mixture was kept at 0 °C overnight and the resulting precipitate was filtered and identified as the E-azlactone **9** (99%).

Scheme 2



a) HBr (g), 48% HBr, 0 °C; b) CH_2N_2, ether, rt; c) DMAP, MeOH, rt, then 0 °C; d) $(Boc)_2O$, 4-pyrrolidinopyridine, THF, rt; e) $NH_2NH_2{\cdot}H_2O$, MeOH, rt; f) HCl, AcOEt, rt; g) i. 1N $LiOH{\cdot}H_2O$, dioxane, rt; ii. Dowex 50X2-200, 10% Py.

The following steps were then effected as detailed for **8**. Treatment of **9** with ethereal diazomethane (**10**, 34%) sequentially followed by methanolysis (**11**, 55%) and treatment with di-t-butyl dicarbonate afforded the N-benzoyl-N-Boc ester derivative **12** in 88% yield. Removal of the N-benzamido (39%) and N-Boc (98%) groups from **12** by hydrazinolysis and acidic treatment, respectively, yielded the cyclopropane ester **14** which was finally submitted to hydrolysis (LiOH, dioxane-water) and chromatography on Dowex 50x2-200 (10% pyridine) to give the (±)-(E)-Δ-Trp (**15**) in 52% yield, with an overall yield from **2** of 8.1%. The evaluation of the biological properties of these new partially constrained tryptophan analogs as well as their introduction in peptidomimetic derivatives is in progress and the results will be reported in due course.

EXPERIMENTAL

General Methods. Melting points were determined by the capillary method on a Büchi 535 electrothermal apparatus and are uncorrected. ^{1}H-NMR spectra were taken on a Bruker AC 200 spectrometer as solutions in $CDCl_3$ unless otherwise indicated. Proton chemical shifts are reported in p.p.m. downfield from tetramethylsilane, except with D_2O which was also used as an internal standard.

GC-Mass spectrometry was performed on a Hewlett-Packard gaschromatograph HP 5890 (column and conditions: HP-1, 12 m, 0.20 mm ID, 0.33 μm f.t., 150(1')/280 °C, 10 °C/min) equipped with a mass detector HP 5971. Combustion analyses were performed on a 1102 Automatic Analyzer Carlo Erba (Italy). Flash chromatography was performed on Merck silica gel (0.040-0.063 mm). Medium pressure chromatography (mpc) was performed on Merck LiChroprep Si 60 and LiChroprep RP-8 (reversed phase) lobar columns.

(Z)-2-Phenyl-4-(N-acetyl-3-indolylmethylene)-oxazol-5-one (2). Indole-3-carboxaldehyde (**1**, 10.0 g, 69 mmol), hippuric acid (12.35 g, 69 mmol) and dry sodium acetate (5.5 g, 69 mmol) were suspended in acetic anhydride and allowed to react under vigorous magnetic stirring at 80 °C for 40 min. Filtration of the warm reaction mixture afforded a crystalline solid which was washed with acetic anhydride (20 ml), water (20 ml) and then dried in high vacuum overnight to give the Z-azlactone **2** (10.23 g, 46%), mp 203-5 °C; ν_{max} ($CHCl_3$) 1785, 1760, 1645 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.80 (3H, s, CH_3CO), 7.35-7.65 and 7.80-8.80 (11H, 2 x m, olefinic CH and aromatics).

(±)-(Z)-2-(N-Acetyl-indol-3-yl)-5-phenyl-6-oxo-4-azaspiro-[2,4]-hept-4-en-7-one (3). A suspension of **2** (10.0 g, 30.3 mmol) in a freshly distilled ethereal solution of diazomethane (500 ml, from 65 g of diazald™) was stirred overnight at room temperature. After filtration of the pale yellow suspension, the filtrate was evaporated to give a brownish oil (8.17 g) which was submitted to flash chromatography: elution with light petroleum-ethyl acetate 8:2 and following re-crystallization from light petroleum-ethyl acetate of the solid thus obtained yielded pure **3** (4.79 g, 46%), mp 141-2 °C; ^{1}H-NMR ($CDCl_3$) δ 2.25-2.40 (2H, m, CH_2), 2.65 (3H, s, CH_3CO), 3.25 (1H, m, cyclopropylic CH), 7.20-7.50 and 7.85-8.45 (10H, 2 x m, aromatics).

(±)-(Z)-Methyl 2-benzamido-2,3-methano-3-(N-acetyl-indol-3-yl)-propanoate (4). DMAP (0.533 g, 4.36 mmol) was added to a suspension of **3** (1.50 g, 4.36 mmol) in anhydrous methanol (60 ml) and the resulting mixture was magnetically stirred at room temperature for 35 min. The pale yellow solid thus formed was then filtered and collected to give **4** (1.40 g, 85.6%), mp 157-9 °C; ^{1}H-NMR ($CDCl_3$) δ 1.81 and 2.43 (2H, 2 x dd, J=5.70 Hz, J=7.85 Hz, J=9.50 Hz, CH_2), 2.52 (3H, s, CH_3CO), 3.01 (1H, qd, J=1.36 Hz, J=7.85 Hz, 3-CH), 3.81 (3H, s, CO_2CH_3), 6.20 (1H, br s, NH), 7.25-8.40 (10H, 2 x m, aromatics); MS *m/e* 377 (M+1), 271, 256.

(±)-(Z)-Methyl 2-(N-benzoyl-N-t-butoxycarbonylamino)-2,3-methano-3-(N-acetyl-indol-3-yl)-propanoate (5). Di-*t*-butyl dicarbonate (1.04 g, 4.78 mmol) and DMAP (0.29 g, 2.39 mmol) were added to a suspension of **4** (0.90 g, 2.39 mmol) in anhydrous dichloromethane (30 ml) and the resulting mixture was kept under magnetic stirring in a nitrogen atmosphere at room temperature for 2 h. After evaporation of the solvent, the crude reaction product (2.28 g) was dissolved in dichloromethane (25 ml), washed with 5% citric acid (2x20 ml), brine (25 ml) and dried over anhydrous sodium sulfate. Evaporation of the solvent

yielded pure **5** (1.08 g, 95%); ^{1}H-NMR ($CDCl_3$) δ 1.55 (9H, br s, *t*-Boc), 2.30 (2H m, CH_2), 2.52 (3H, s, CH_3CO), 3.40 (1H, m, 3-CH), 3.80 (3H, s, CO_2CH_3), 7.00-8.50 (10H, 4 x m, aromatics).

(±)-(Z)-Methyl 2-t-butoxycarbonylamino-2,3-methano-3-(indol-3-yl)-propanoate (6). Hydrazine monohydrate (10.32 g, 206 mmol) was added to a magnetically stirred suspension of **5** (1.0 g, 2.10 mmol) in anhydrous methanol (60 ml) at room temperature. Stirring was continued for 1 h after which the solvent was evaporated on a rotary evaporator, taking care in the control of the water bath (below 30 °C). The residue (1.2 g) was then submitted to flash chromatography: elution with chloroform-methanol 95:5 afforded **6** (0.60 g, 86.6); ^{1}H-NMR ($CDCl_3$) δ 1.35 (9H, br s, *t*-Boc), 1.65 and 2.22 (2H, 2 x m, CH_2), 2.95 (1H, m, 3-CH), 3.80 (3H, s, CO_2CH_3), 6.95 (1H, br s, NHCO), 7.10-7.60 (5H, 3 x m, aromatic's), 8.25 (1H, br s, NH).

(±)-(Z)-Methyl 2-amino-2,3-methano-3-(indol-3-yl)-propanoate (7). 12N Hydrochloric acid (2 ml) was added to a solution of **6** (0.30 g, 0.91 mmol) in ethyl acetate (10 ml) and the resulting mixture was magnetically stirred at room temperature for 30 min. The reaction mixture was then neutralized with saturated sodium hydrogen carbonate, the organic phase separated and the aqueous layer was extracted with ethyl acetate (2x10 ml). The combined organic phases were washed with brine (20 ml) and dried over anhydrous sodium sulfate. Evaporation of the solvent gave **7** as an orange oil (0.205 g, 98%); ^{1}H-NMR ($CDCl_3$) δ 1.45 and 1.90 (2H, 2 x m, CH_2), 2.56 (1H, m, 3-CH), 3.80 (3H, s, CO_2CH_3), 6.90-7.60 (5H, m, aromatic's), 8.45 (1H, br s, NH).

(±)-(Z)-2-Amino-2,3-methano-3-(indol-3-yl)-propanoic acid (8). 1N Lithium hydroxide monohydrate (3 ml) was added to a solution of **7** (0.47 g, 2.04 mmol) in dioxane (6 ml) and the resulting mixture was kept under magnetic stirring at room temperature overnight. The reaction mixture was then evaporated to dryness, the residue diluted with water and neutralized with 1N hydrochloric acid. Ion exchange resin chromatography on Dowex 50x2 200 and elution with 10% pyridine yielded **8** (0.114 g, 26%), mp 179-181 °C; ^{1}H-NMR (D_2O) δ 1.58 and 1.80 (2H, 2 x dd, J=7.7 Hz, J=9.6 Hz, CH_2), 2.82 (1H, qd, J=1.1 Hz, J=7.7 Hz, 3-CH), 7.00-7.50 (6H, 2 x m, aromatics). Anal. Calcd. for $C_{12}H_{12}N_2O_2$: C, 66.65; H, 5.59; N, 12.95. Found: C, 66.60; H, 5.62; N, 12.90.

(E)-2-Phenyl-4-(N-acetyl-3-indolylmethylene)-oxazol-5-one (9). HBr was bubbled for 1.5 h into a magnetically stirred suspension of **2** (15.0 g, 45.5 mmol) in 48% HBr (250 ml) kept at 0 °C. The resulting solution was maintained at 0 °C for 12 h after which the formation of a solid was observed. The reaction mixture was then poured into iced water, the solid was filtered and dried at 40 °C for 12 h to give **9** (14.9 g, 99%), mp 191-3 °C; ^{1}H-NMR ($CDCl_3$) δ 2.80 (3H, s, $COCH_3$), 7.35-7.80 and 8.00-8.55 (10H, 2xm, aromatics), 9.35 (1H, s, N-CH=C).

(±)-(E)-2-(N-Acetyl-indol-3-yl)-5-phenyl-6-oxo-4-azaspiro-[2,4]-hept-4-en-7-one (10). A suspension of **9** (1.0 g, 3.03 mmol) in a freshly distilled ethereal solution of diazomethane (54 ml, from 8.78 g of diazald™) was stirred overnight at room temperature. After filtration of the pale yellow suspension, the filtrate was evaporated and the residue (0.9 g) was submitted to flash chromatography: elution with light petroleum-ethyl acetate 8:2 and re-crystallization from light petroleum-ethyl acetate of the solid thus obtained yielded pure **10** (0.850 g, 81%), mp 160-3 °C; ^{1}H-NMR ($CDCl_3$) δ 2.10-2.25 and 2.35-2.52 (2H, 2xm, CH_2), 2.60 (3H, s, CH_3CO), 3.30-3.50 (1H, m, cyclopropylic CH), 7.10-7.50 and 7.85-7.95 (9H, 2 x m, aromatics), 8.30 (1H, d, J=7.8 Hz, aromatic).

(±)-(E)-Methyl 2-benzamido-2,3-methano-3-(N-acetyl-indol-3-yl)-propanoate (11). DMAP (0.355 g, 2.91 mmol) was added to a suspension of **10** (1.0 g, 2.91 mmol) in anhydrous methanol (77 ml) and the resulting mixture was magnetically stirred at room temperature for 90 min and then at 0 °C for 30 min. The pale yellow solid thus formed was then filtered and collected to give **11** (0.602 g, 55%), mp 261-3 °C; ^{1}H-NMR ($CDCl_3$) δ 1.86 and 2.31 (2H, 2 x dd, J=5.71 Hz, J=9.70 Hz, J=8.30 Hz, CH_2), 2.64 (3H, s, CH_3CO), 2.90 (1H, qd, J=1.34 Hz, J=8.3 Hz, 3-CH), 3.41 (3H, s, CO_2CH_3), 6.83 (1H, s, NH), 7.25-8.40 (10H, 4 x m, aromatics); MS *m/e* 377 (M+1), 271, 256.

(±)-(E)-Methyl 2-(N-benzoyl-N-t-butoxycarbonylamino)-2,3-methano-3-(N-acetyl-indol-3-yl)-propanoate (12). Di-*t*-butyl dicarbonate (5.81 g, 26.6 mmol) and 4-pyrrolidinopyridine (0.065 g, 0.44 mmol) were added to a suspension of **11** (1.0 g, 2.66 mmol) in anhydrous THF (40 ml) and the resulting mixture was kept under magnetic stirring in a nitrogen atmosphere at room temperature for 22 h. After evaporation of the solvent, the crude reaction product was dissolved in dichloromethane (200 ml) and washed with 5% citric acid (4x60 ml). The aqueous layer was then extracted with dichloromethane (2x70 ml) and the combined organic phases were washed with brine (100 ml) and dried over anhydrous sodium sulfate. Evaporation of the solvent yielded a residue which was submitted to flash chromatography: elution with light petroleum-AcOEt (8:2) afforded **12** (1.18 g, 93%); ^{1}H-NMR ($CDCl_3$) δ 1.15 (9H, br s, *t*-Boc), 2.35 (2H m, CH_2), 2.57 (3H, s, CH_3CO), 3.15 (1H, m, 3-CH), 3.40 (3H, s, CO_2CH_3), 7.20-8.40 (10H, 4 x m, aromatics).

(±)-(E)-Methyl 2-t-butoxycarbonylamino-2,3-methano-3-(indol-3-yl)-propanoate (13). Hydrazine monohydrate (7.14 g, 142.6 mmol) was added to a magnetically stirred suspension of **12** (0.80 g, 1.68 mmol) in anhydrous methanol (40 ml) at room temperature. Stirring was continued for 15 min after which the solvent was evaporated on a rotary evaporator, taking care in the control of the water bath (below 30 °C). The residue (1 g) was dissolved in chloroform (150 ml) and washed with 5% citric acid (4x50 ml). The aqueous layer was then extracted with chloroform (2x50 ml) and the combined organic phases were washed with brine (100 ml) and dried over anhydrous sodium sulfate. Evaporation of the solvent yielded a residue which was submitted to flash chromatography: elution with chloroform-methanol (99.9:0.1) gave **13** (0.216 g, 39%); ^{1}H-NMR ($CDCl_3$) δ 1.50 (9H, br s, *t*-Boc), 1.70 and 2.20 (2H, 2 x m, CH_2), 2.80 (1H,

m, 3-CH), 3.35 (3H, s, CO_2CH_3), 6.95 (1H, br s, NHCO), 7.10-7.75 (5H, 3 x m, aromatic's), 8.70 (1H, s, NH).

(±)-(E)-Methyl 2-amino-2,3-methano-3-(indol-3-yl)-propanoate (14). 12N Hydrochloric acid (2 ml) was added to a suspension of **13** (0.150 g, 0.45 mmol) in ethyl acetate (5 ml) and the resulting mixture was magnetically stirred at room temperature for 30 min. The reaction mixture was then neutralized with saturated sodium hydrogen carbonate, the organic phase separated and the aqueous layer was extracted with ethyl acetate (2x10 ml). The combined organic phases were washed with brine (20 ml) and dried over anhydrous sodium sulfate. Evaporation of the solvent gave **14** as a reddish oil (0.124 g, 98%); ^{1}H-NMR ($CDCl_3$) δ 1.25 and 1.85 (2H, 2 x m, CH_2), 2.78 (1H, m, 3-CH), 3.70 (3H, s, CO_2CH_3), 6.90-7.50 (5H, m, aromatic's), 8.20 (1H, br s, NH).

(±)-(E)-2-Amino-2,3-methano-3-(indol-3-yl)-propanoic acid (15). 1N Lithium hydroxide monohydrate (0.74 ml) was added to a solution of **14** (0.100 g, 0.44 mmol) in dioxane (1.5 ml) and the resulting mixture was kept under magnetic stirring at room temperature for 12 h. The reaction mixture was then evaporated to dryness, the residue diluted with water and neutralized with 1N hydrochloric acid. Ion exchange resin chromatography on Dowex 50x2 200 and elution with 10% pyridine yielded **15** (0.050 g, 52%), mp 137-139 °C; ^{1}H-NMR (D_2O) δ 1.48 and 1.75 (2H, 2 x dd, J=7.0 Hz, J=9.6 Hz, CH_2), 2.78 (1H, dd, J=8.6 Hz, J=9.5 Hz, 3-CH), 6.95-7.50 (6H, 3 x m, aromatics). Anal. Calcd. for $C_{12}H_{12}N_2O_2$: C, 66.65; H, 5.59; N, 12.95. Found: C, 66.62; H, 5.59; N, 12.92.

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