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Preliminary communication

# Dimers from dechlorinated rebeccamycin: synthesis, interaction with DNA, and antiproliferative activities

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**Abstract**

In the course of structure–activity relationships on rebeccamycin analogues, two dimers of dechlorinated rebeccamycin were synthesised with the aim to improve the interaction with DNA and in vitro antiproliferative activities. The synthesis of two dimeric compounds obtained by joining two molecules of dechlorinated rebeccamycin via the imide nitrogen is described. Melting temperature and DNase I footprinting studies were performed to investigate their interaction with DNA. Four tumour cell lines, murine L1210 leukaemia, human HT29 colon carcinoma, A549 non-small cell lung carcinoma and K-562 leukaemia, were used to evaluate the cytotoxicity of the drugs. Their effects on the cell cycle of L1210 cells were also investigated. 

*Keywords:* Rebeccamycin; Dimers; Antitumour agents; DNA intercalators

## 1. Introduction

Indolocarbazoles compounds bearing a sugar moiety represent a promising class of antitumour agents [1–3]. The protein kinase C inhibitors UCN-01, CGP 41251, and CEP-751 analogues of staurosporine, and the topoisomerases inhibitors NB-506, J-107088, and NCS # 655649, structurally related to rebeccamycin, have been tested clinically or are currently undergoing clinical trials (Fig. 1) [4–6].

We have shown in previous works that rebeccamycin analogues bind to DNA and inhibit topoisomerase I [7–10]. The planar indolocarbazole chromophore can intercalate in DNA locating the carbohydrate residue in one of the two helical grooves as is the case with many DNA binding antibiotics including the anthracyclines. The design of dimeric compounds represents an attractive strategy to increase both DNA binding affinities and sequence selectivity. In the past, numerous bis-intercalating agents including diacridines, bis-anthracyclines, bis-naphtalimides and the major groove binding drug ditercalinium, have been successfully designed [11–17]. In most cases, these compounds contain two planar chromophores separated by an aminoalkyl linker of variable length, usually 10–12 Å long. The connecting chain frequently incorporate secondary amines (e.g. –NH–$(CH_2)_3$–NH–$(CH_2)_4$–NH–$(CH_2)_3$–NH–) that may be protonated at neutral pH and can enhance water solubility and reinforce DNA binding. But the two moieties can also be associated via a rigid linker containing an aromatic system (–NH–$CH_2$–$C_6H_4$–$C_2$–M–) (Fig. 2). This is the case for the bis-daunomycin analogues WP631 and WP652 (Fig. 2) which have shown exceptional DNA binding and anticancer activities [14,18].

These considerations prompted us to undertake the synthesis of indolocarbazole dimers incorporating two dechlorinated rebeccamycin moieties. We have shown previously that the chlorine atoms at positions 1 and 11 in rebeccamycin are detrimental to the formation of

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DNA intercalation complexes [19]. In both cases, the two indolocarbazole chromophores were connected via their nitrogen imide on the F ring, using a flexible linker, $-(CH_2)_2-NH-(CH_2)_3-NH-(CH_2)_2-$ or a more rigid connector, $-NH-CH_2-C_6H_4-CH_2-NH$. Here we describe the synthesis of the two drugs (Fig. 3) and preliminary data concerning their interaction with DNA and biological activities.

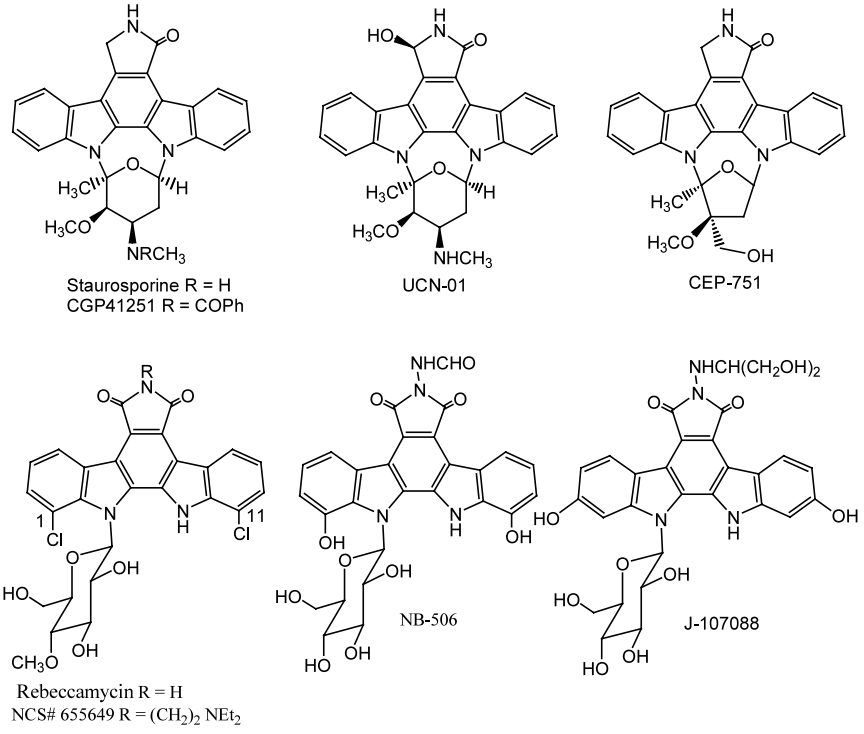


Fig. 1. Indolocarbazole protein kinase C and topoisomerase I inhibitors.

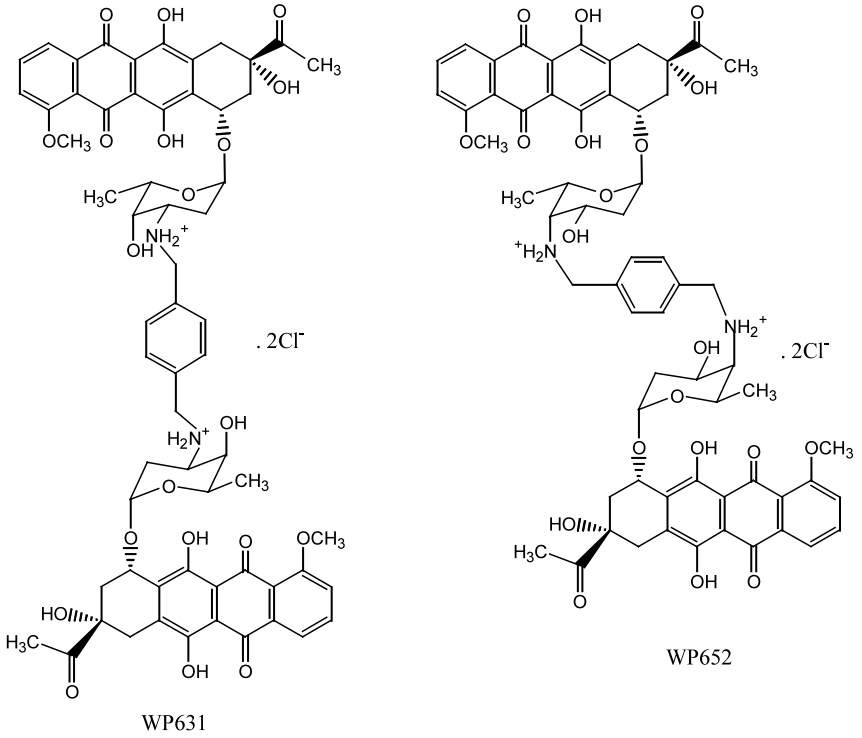


Fig. 2. Dimers from daunomycin.

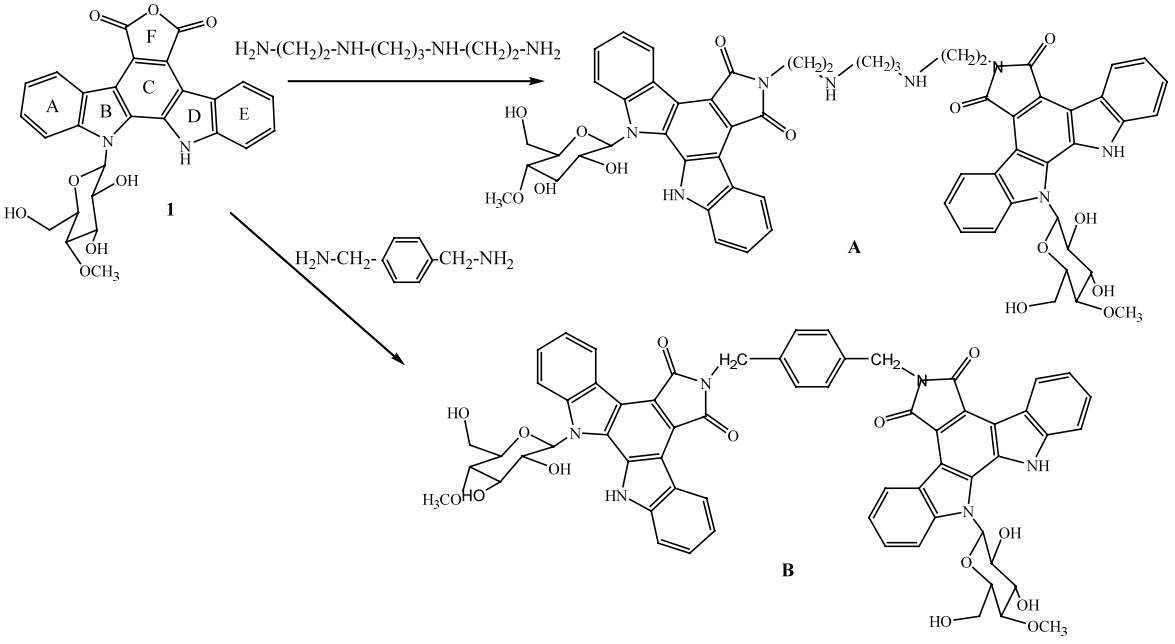


Fig. 3. Synthesis of dimers from rebeccamycin.

## 2. Results and discussion

Compound **A** and **B** were prepared from dechlorinated anhydride **1** [20] by reaction with *N*,*N*′-bis(2-aminoethyl)-1,3-propanediamine and *p*-xylylene diamine, respectively, (Fig. 3), according to a method described for the synthesis of bis-naphtalimides [21].

### 2.1. DNA interaction

Melting temperature (*T*m) measurements performed with calf thymus DNA and the polynucleotide poly(-dAT)$_2$ indicate that the dimeric compound **A** exhibits a higher affinity for DNA compared with dechlorinated rebeccamycin whereas the dimeric compound **B** shows no effect on the *T*m of nucleic acids (Fig. 4). The rigid linker containing the aromatic ring is probably too short to enable bis-intercalation. On the contrary, the aminoalkyl linker seems to be well adapted to reinforce drug-DNA interaction. It must be noted, however, that the enhanced interaction of compound **A** with DNA is detrimental to topoisomerase I inhibition. No stimulation of topoisomerase I-mediated DNA cleavage was observed with the two dimers. The sequence selectivity was investigated by DNase I footprinting. Compound **B** gave no footprint as it is the case with dechlorinated rebeccamycin. But an interesting effect was observed with dimer **A** containing the aminoalkyl linker. As shown in Fig. 5, three regions show attenuated cleavage by the nuclease in the presence of compound **A**: 5′-CAGT, 5′-TAAT and 5′-ACTATAG which correspond to preferential drug binding sites. Apparently, this compound exhibits an unusual AT preference. Additional biophysical and biochemical techniques are being used to investigate further its DNA binding properties.

### 2.2. Cytotoxicity

The cytotoxic activity of dimers **A** and **B** was evaluated towards four tumour cell lines, murine L1210 leukaemia, human HT29 colon carcinoma, A549 non-small cell lung carcinoma and K-562 leukaemia. The parent compound, dechlorinated rebeccamycin, was used as a reference and the cytotoxicity measurements were performed using a conventional microculture tetrazolium assay [22]. The results are expressed as $IC_{50}$ (μM) which refers to the concentration required to reduce by 50% the optical density of treated cells with respect to untreated controls. In parallel, a cell cycle analysis was performed using the L1210 leukaemia cell line [23] (Table 1).

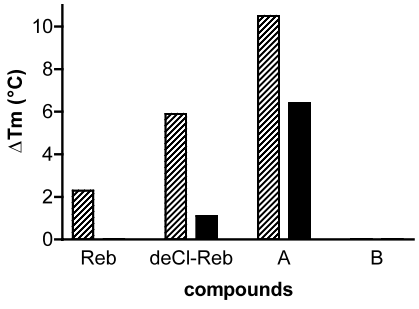


Fig. 4. Variation of the Δ*T*m ($Tm^{drug-DNA\ complex} - Tm^{DNA\ alone}$, in °C) of the complexes between calf thymus DNA and the test compounds. Melting temperature measurements were performed in BPE buffer at pH 7.1 with a drug/DNA ratio of 0.1.

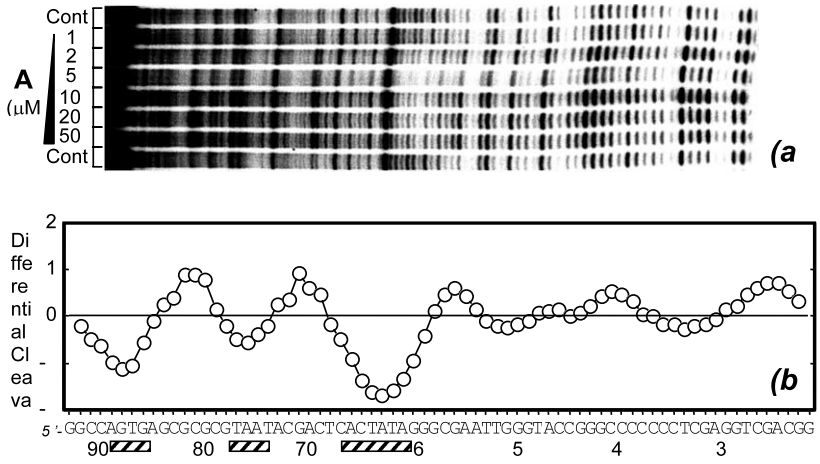


Fig. 5. Sequence selective binding of the drugs to DNA. (a) The gel shows DNase I footprinting with the 3′-end 174-mer *Pvu*II-*Eco*RI fragment from plasmid pKS. The products of nuclease digestion were resolved on an 8% polyacrylamide gel containing 8 M urea. Control tracks (marked Cont) contained no drug. (b) The differential cleavage plot shows the susceptibility of the DNA fragment to DNase I cutting in the presence of 20 μM compound **A**. Negative values correspond to a ligand-protected site and positive values represent enhanced cleavage. Vertical scales are in units of ln(*f*a)–ln(*f*c), where *f*a is the fractional cleavage at any bond in the presence of the drug and *f*c is the fractional cleavage of the same bond in the control, given closely similar extents of overall digestion. Only the region of the restriction fragment analysed by densitometry is shown. Dashed boxes indicate the positions of inhibition of DNase I cutting (drug binding sites).

Table 1
In vitro antiproliferative activities against four tumor cell lines: murine leukaemia L1210, and human HT29 colon carcinoma, A549 non-small cell lung carcinoma and K-562 leukaemia ($IC_{50}$ μM). Effect on LT1210 cell cycle: percentage of cells recoverd in the G2+M phases with a concentration of drug expressed in μM

| Cpd | L1210 cell cycle effect [a] | L1210 | HT29 | A549 | K-562 |
|---|---|---|---|---|---|
| Dechlor reb | G2M 71% at 1 μM | 0.11 | 2.5 | 2 | <0.1 |
| 1 | ne | 70.3 | ~10 | >10 | >10 |
| **A** | G2M 65% at 5 μM | 0.9 | 0.5 | 0.4 | ~0.1 |
| **B** | ne | 14 | 32 | 23 | ne |

ne: Not evaluated.
[a] Twenty-four percent of untreated control cells were recovered in the G2+M phase of the cell cycle.

## 3. Conclusion

This is the first report on rebeccamycin dimers. The linker between the two chromophores must be long enough to enable the two planar chromophores to interact with DNA. This seems to be the case for compound **A** but not compound **B**. Further dimers containing an aminoalkyl connector are being synthesised.

## 4. Experimental

### 4.1. Chemistry

IR spectra were recorded on a Perkin–Elmer 881 spectrometer ($\nu$ in $cm^{-1}$). NMR spectra were performed on a Bruker AC 400 ($^1H$: 400 MHz, $^{13}C$: 100 MHz) (chemical shifts $\delta$ in ppm, the following abbreviations are used: singlet (s), broad singlet (br s), doublet (d), triplet (t), multiplet (m), tertiary carbons (C tert), quaternary carbons (C quat). The signals were assigned from $^1H$–$^1H$ COSY and $^{13}C$–$^1H$ correlations. Mass spectra (FAB+) were determined at CESAMO (Talence, France) on a high resolution Fisons Autospec-Q spectrometer, and at Clermont-Ferrand (France) on a Hewlett–Packard 5989B (electrospray 59987A) Chromatographic purifications were performed by flash silicagel Geduran SI 60 (Merck) 0.040–0.063 mm or Kieselgel 60 (Merck) 0.063–0.200 mm column chromatography. For purity tests, TLC was performed on fluorescent silica gel plates (60 $F_{254}$ from Merck). Rebeccamycin was from our laboratory stock sample.

#### 4.1.1. *Di-ethyl-2-[6-(12-(4-O-methyl-β-D-glucopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-a]pyrrolo[3,4-c]-carbazole-5,7-dione)]-1,3-propanediamine* ***A***

To a solution of anhydride **1** (50 mg, 0.099 mmol) in THF (5 mL) was added dropwise *N*,*N*′-bis(2-aminoethyl)-1,3-propanediamine (8 μL, 0.045 mmol). The mixture was refluxed for 4 days with light protection. After cooling, water and EtOAc were added. The

pH was adjusted to pH 12 with saturated aqueous $NaHCO_3$. After extraction with EtOAc, the organic phase was dried over $MgSO_4$ and the solvent was removed. The residue was purified by flash chromatography (eluent, EtOAc–cyclohexane 8:2) to give the free amine **A** (43.7 mg, 0.038 mmol, 86% yield) as a yellow solid. M.p. 280 °C. IR (KBr) $\nu_{C=O}$ 1695, 1750 $cm^{-1}$, $\nu_{NH,OH}$ 3200–3600 $cm^{-1}$. Mass (electrospray) $[M+Na]^+$ Calc. for $C_{61}H_{60}N_8O_{14}$ Na 1141 Found: 1141. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.71 (6H, s, $OCH_3$), 3.59–4.08 (26H, m), 5.20 (2H, br s, OH), 5.36 (2H, br s, OH), 6.23 (2H, br s, OH), 6.35 (2H, d, $J = 8.9$ Hz, $H_{1'}$), 7.41 (4H, t, $J = 7.5$ Hz), 7.62 (4H, t, $J = 7.5$ Hz), 7.76 (2H, dd, $J_1 = 8.1$ Hz, $J_2 = 3.7$ Hz), 8.00 (2H, dd, $J_1 = 8.5$ Hz, $J_2 = 4.7$ Hz), 9.16 (2H, d, $J = 7.8$ Hz), 9.22 (2H, d, $J = 7.5$ Hz), 11.68 (2H, s, $N_{indole}$–H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$ 40.6, 40.2 (6C) ($CH_2$), 58.5 (2C) ($C_{6'}$), 60.1 (2C) ($OCH_3$), 73.1 (2C), 76.3 (2C), 77.1 (2C), 77.3 (2C), 84.2 (2C) ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.8 (2C), 112.2 (2C), 120.5 (2C), 120.7 (2C), 124.4 (4C), 126.9 (2C), 127.1 (2C) (C tert arom), 117.1 (2C), 118.3 (2C), 118.6 (2C), 119.9 (2C), 121.0 (2C), 121.3 (2C), 128.2 (2C), 129.6 (2C), 140.9 (2C), 142.2 (2C) (C quat arom), 169.5 (2C), 169.6 (2C) (C=O).

#### *4.1.2. 1,4-Di[6-(12-(4-O-methyl-β-D-glucopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-a]pyrrolo[3,4-c]-carbazole-5,7-dione)]methyl benzene* ***B***

To a solution of anhydride **1** (63 mg, 0.124 mmol) in ethanol (5 mL) was added dropwise commercial *p*-xylenediamine (8 mg, 0.059 mmol). The light protected mixture was refluxed for 13 days. After cooling, a mixture of water–EtOAc was added. The pH was adjusted to pH 4 by addition of aqueous HCl. After extraction with EtOAc, the organic phase was dried over $MgSO_4$ and the solvent was removed. The residue was purified by flash chromatography using silicagel neutralised with $NEt_3$ (eluent, EtOAc) to give **B** (56 mg, 0.051 mmol, 86% yield) as a yellow–orange solid. M.p. > 300 °C. IR (KBr) $\nu_{C=O}$ 1700, 1760 $cm^{-1}$, $\nu_{NH,OH}$ 3200–3600 $cm^{-1}$. HRMS (FAB+) $[M+H]^+$ Calc. for $C_{62}H_{53}N_6O_{14}$ 1105.3619 Found: 1105.3591. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.53–3.59 (2H, m), 3.69 (6H, s, $OCH_3$), 3.73–3.83 (4H, m), 3.86–3.93 (2H, m), 3.96–4.08 (4H, m), 4.95 (2H, s, $2CH_2$), 4.97 (2H, d, $J = 5.9$ Hz, 2OH), 5.32 (2H, d, $J = 5.7$ Hz, 2OH), 6.20 (2H, br s, 2OH), 6.32 (2H, d, $J = 8.9$ Hz, $2H_{1'}$), 7.38 (4H, t, $J = 7.6$ Hz), 7.48 (4H, s), 7.61 (4H, t, $J = 7.5$ Hz), 7.74 (2H, d, $J = 8.2$ Hz), 7.99 (2H, d, $J = 8.5$ Hz), 9.09 (2H, d, $J = 8.0$ Hz), 9.16 (2H, d, $J = 7.9$ Hz), 11.66 (2H, $2N_{indole}$–H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$ 40.5 (N–$CH_2$), 58.5 ($C_{6'}$), 60.1 ($OCH_3$), 65.3 ($NCH_2$), 73.1, 76.3, 77.1, 77.3, 84.1 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 117.1, 118.1, 118.7, 119.7, 120.9, 121.3, 128.3, 129.7, 136.6, 140.8, 142.2 (C quat arom), 111.9, 112.2, 120.5, 120.7, 124.3, 126.9, 127.1, 127.9 (C tert arom), 169.2, 169.3 (C=O).

### *4.2. Biochemical studies*

Melting curves were measured using an Uvikon 943 spectrophotometer coupled to a Neslab RTE111 cryostat. For each series of measurements, 12 samples were placed in a thermostatically controlled cell-holder, and the quartz cuvettes (10 mm pathlength) were heated by circulating water. Measurements were performed in BPE buffer pH 7.1 (6 mM $Na_2HPO_4$, 2 mM $NaH_2PO_4$, 1 mM EDTA). The temperature inside the cuvette was measured with a platinum probe; it was increased over the range 20–100 °C with a heating rate of 1 °C $min^{-1}$. The 'melting' temperature *T*m was taken as the mid-point of the hyperchromic transition. DNase I footprinting experiments were performed as previously described [24]. Briefly, reactions were conducted in a total volume of 8 μL. Samples (2 μL) of the labelled DNA fragment were incubated with 4 μL of the buffer solution containing the ligand at appropriate concentration. After 30 min incubation at 37 °C, the digestion was initiated by the addition of 2 μL of a DNase I solution. After 3 min, the digestion was stopped by freeze drying, samples were lyophilised, washed once with 50 μL of water, lyophilised again and then resuspended in 4 μL of an 80% formamide solution containing tracking dyes. Samples were heated at 90 °C for 4 min and chilled in ice for 4 min prior to polyacrylamide gel electrophoresis under denaturing conditions (0.3 mm thick, 8% acrylamide gels containing 8 M urea).

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