CERTIFICATE OF ANALYSIS

trans-ZEATIN-9-GLUCURONIDE (Z9GN)

6-((E)-4-hydroxy-3-methylbut-2-enylamino))-9-β-D-glucuronic acid, sodium salt

$C_{16}H_{21}N_5O_7$, MW = 395.38

OLCHEMIM CODE: 001 596n
OLCHEMIM BATCH: Z9GN-A
MANUFACTURING DATE: April 2025
EXPIRATION DATE: April 2035
CAS: 1402800-77-4
BATCH SIZE: 400 MG

SYNTHESIS

Prepared by the condensation of acetobromoglucuronic acid, methyl ester and *trans*-zeatin in dry DMF. Purified by reverse-phase chromatography and re-crystallised from methanol and EtOAc. A white, amorphous solid, soluble in water or methanol. Best solvent dimethyl sulfoxide (10 mg/ml).

ANALYSIS

TLC (silica - chloroform/MeOH. 1/1), *trans*-zeatin-9-β-D-glucuronic acid, sodium salt at 30, 50 and 150 µg loading showed a single spot (Rf 0.17) with no detectable impurities. No tZ (Rf 0.66) or 6-chloropurine (Rf 0.70) detectable. Overall purity 98+%. *trans*-Zeatin content below detection level of 0.1%.

MASS SPETROMETRY: MASS SPETROMETRY: Matches the reference spectrum $[M+H]^+$ = 394.40 amu, ZMD 2000 (Waters) single quadrupole electrospray MS.

HPLC: Samples were dissolved in mobile phase (initial conditions) and separated on RP-column (150 mm × 4.6 mm, 5µm) (Microsorb C18; Varian). The elution was performed with a methanolic gradient (10 mM HCOOH). The flow rate was 0.6 ml/min. The UV absorbance was scanned between 240 – 300 nm. Rt (Z9G) = 7.6 min. Purity > 98+%.

GUS HYDROLYSIS: Zeatin-9-β-D-glucuronic acid, Na salt (1 mg) was incubated with bacterial β−glucuronidase (Sigma type G-8271) in 50 mM sodium phosphate buffer at pH 7.0 at 37°C for 4 h. TLC (silica - chloroform/MeOH, 1/1) of 50 µg showed complete conversion to *trans*-zeatin. The identity of *trans*-zeatin in the enzyme hydolysate was confirmed by co-chromatography of a 1:1 mix of the hydrolysate and authentic *trans*-zeatin in the TLC solvent. Not tested in 3 different solvent systems as stated in data sheet.

STORAGE & STABILITY: ACID LABILE! HYGROSCOPIC!
Store over silica gel in tightly sealed container at –18°C.

FOR LABORATORY USE BY EXPERIENCED PERSONNEL ONLY; NOT FOR DRUG, HOUSEHOLD OR OTHER USES

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