

## The Semi-Synthetic Production of Human Erythropoietin

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Erythropoietin (EPO) is a glycoprotein of 166 amino acids containing 3 *N*-linked carbohydrate groups (at Asparagines 24, 38 and 83) and a single *O*-linked group at Serine 126. The attached carbohydrate moieties serve to prolong circulating half-life and subsequent activity *in vivo*. EPO is produced in the body in response to hypoxia and stimulates the maturation of erythrocytes (red blood cells) from erythroid progenitor cells. Consequently, purified formulations of human EPO are routinely administered in the clinical setting to treat anaemia resulting from HIV infection, cancer and renal failure. Presently, commercial methods of EPO production rely upon large-scale eukaryotic tissue culture systems that are both inefficient and costly. Critically, the EPO produced using this approach exists as a heterogeneous mixture of glycosylated forms, much of which exhibits limited biological activity *in vivo*.

Here we present a novel semi-synthetic strategy for the assembly of human EPO that relies upon the chemical synthesis of short (28 and 32 amino acid) N-terminal regions that contain unique acetylenated cysteine derivatives. Protein ligation between these chemically derived fragments and larger bacterially expressed C-terminal fragments result in a semi-synthetic full length EPO protein amenable to site-specific *in vitro* glycosylation with chemically defined sugars and oligosaccharide mimics, thereby circumventing the molecular heterogeneity associated with natural glycosylation processes.