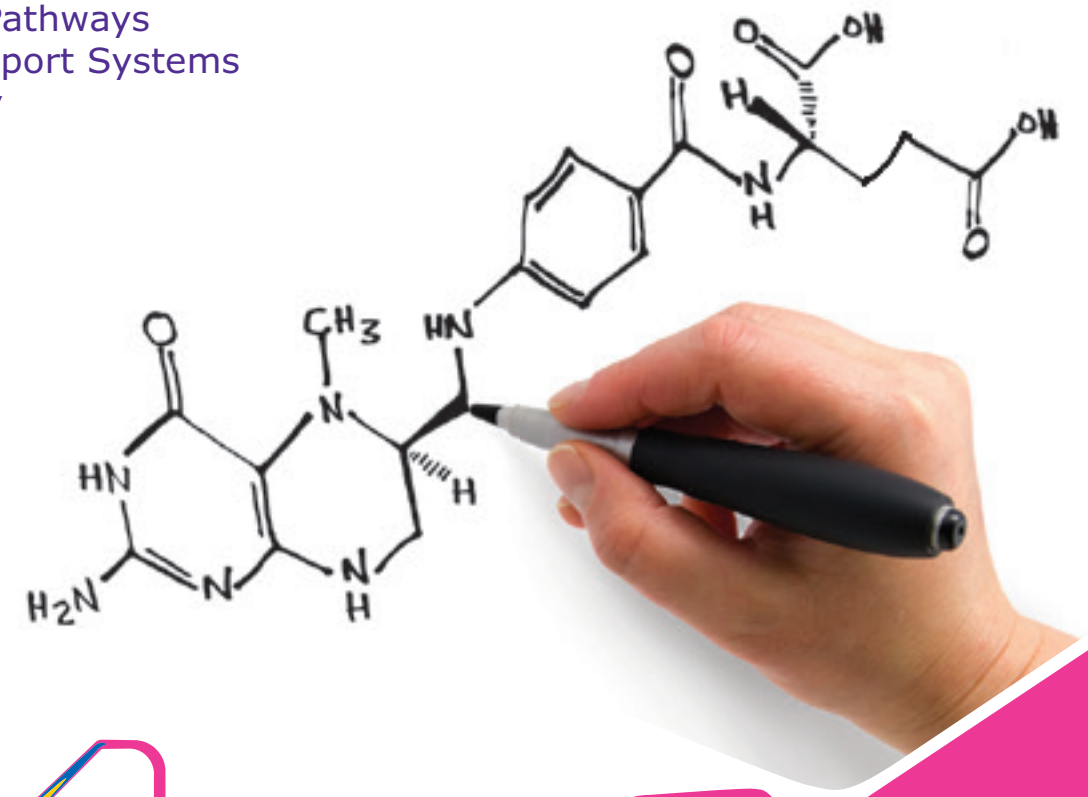
# Folate Compounds
# From The Leading Folate Chemistry Experts

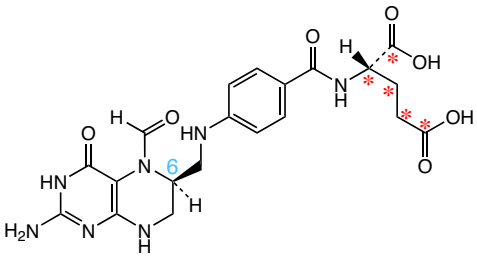
Folate Substance List
Natural Folates
Biochemical Pathways
Cellular Transport Systems
Folate History



The life science business of Merck operates as MilliporeSigma in the U.S. and Canada.

SAFC®
Pharma & Biopharma Raw Material Solutions

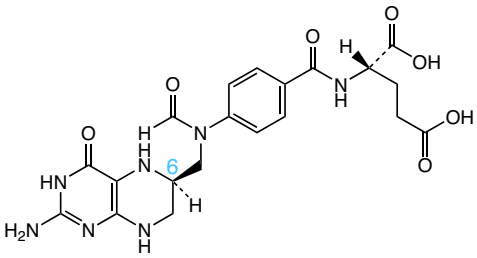
# Folate Substance List



**5-Formyltetrahydrofolate** (Leucovorin)

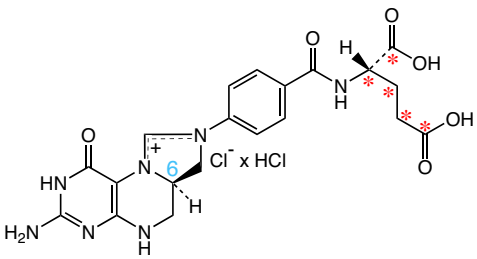
$C_{20}H_{23}N_7O_7$ (6R,S)-, (6R)-, (6S)-5-CHO-$H_4$PteGlu,
MW 473.44 Ca- or $Na_2$-salt

$C_{15}{}^{13}C_5H_{23}N_7O_7$ (6S)-5-CHO-$H_4$Pte[$^{13}C_5$]Glu, Ca-salt
MW 478.44



**10-Formyltetrahydrofolate**

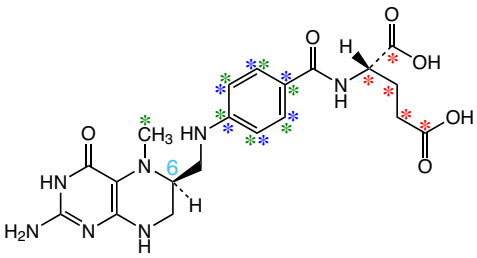
$C_{20}H_{23}N_7O_7$ (6R,S)-, (6S)-, (6R)-10-CHO-$H_4$PteGlu,
MW 473.44 Ca- or $Na_2$-salt



**5,10-Methenyltetrahydrofolate** (Anhydroleucovorin)

$C_{20}H_{23}Cl_2N_7O_6$ (6R,S)-, (6R)-, (6R)-5,10-$CH^+$-$H_4$PteGlu-
MW 528.35 Cl x HCl (Cl x HCl salt)

$C_{15}{}^{13}C_5H_{23}Cl_2N_7O_6$ (6R)-5,10-$CH^+$-$H_4$Pte[$^{13}C_5$]Glu-
MW 533.35 Cl xHCl



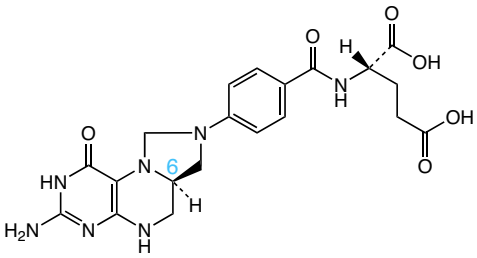
**5-Methyltetrahydrofolate**

$C_{20}H_{25}N_7O_6$ (6R,S)-, (6R)-, (6S)-5-$CH_3$-$H_4$PteGlu,
MW 459.46 Ca- or $Na_2$-salt

$C_{15}{}^{13}C_5H_{25}N_7O_7$ (6S)-5-$CH_3$-$H_4$Pte[$^{13}C_5$]Glu, Ca-salt
MW 464.46

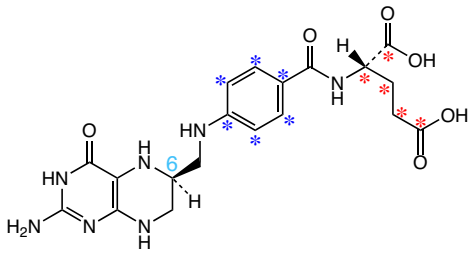
$C_{13}{}^{13}C_6H_{25}N_7O_7$ (6S)-5-$CH_3$-$H_4$[$^{13}C_6$]PteGlu, Ca-salt
MW 465.46

$C_{13}{}^{13}C_7H_{25}N_7O_6$ (6S)-5-$^{13}CH_3$-$H_4$[$^{13}C_6$]PteGlu, Ca-salt
MW 466.46



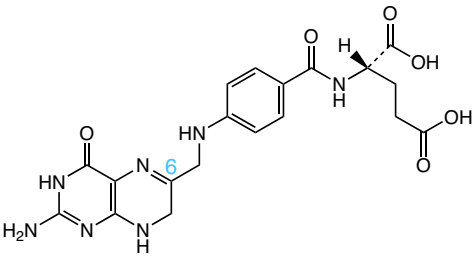
**5,10-Methylenetetrahydrofolate**

$C_{20}H_{23}N_7O_6$ (6R,S)-, (6S)-, (6R)-5,10-$CH_2$-$H_4$PteGlu,
MW 457.44 Ca- or $Na_2$-salt



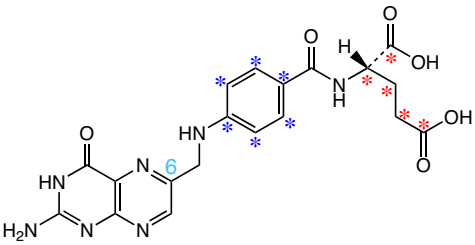
## Tetrahydrofolate

| | |
|---|---|
| $C_{19}H_{23}N_7O_6$<br>MW 445.43 | (6R,S)-, (6R)-, (6S)-$H_4$PteGlu,<br>Ca- or $Na_2$-salt |
| $C_{14}{}^{13}C_5H_{23}N_7O_6$<br>MW 450.43 | (6S)-$H_4$Pte[$^{13}C_5$]Glu, free acid form |
| $C_{13}{}^{13}C_6H_{23}N_7O_6$<br>MW 451.43 | (6S)-$H_4$[$^{13}C_6$]PteGlu, free acid form |



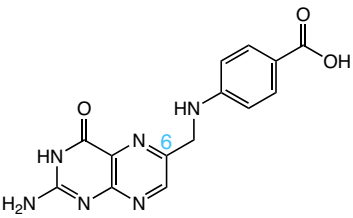
## 7,8-Dihydrofolate

| | |
|---|---|
| $C_{19}H_{21}N_7O_6$<br>MW 443.41 | 7,8-$H_2$PteGlu, free acid form |



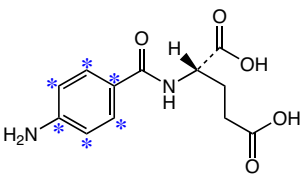
## Folic acid (Pteroylglutamic acid)

| | |
|---|---|
| $C_{19}H_{19}N_7O_6$<br>MW 441.40 | PteGlu, free acid form, $Na_2$-salt |
| $C_{14}{}^{13}C_5H_{19}N_7O_6$<br>MW 446.40 | Pte[$^{13}C_5$]Glu, free acid form |
| $C_{13}{}^{13}C_6H_{19}N_7O_6$<br>MW 447.40 | [$^{13}C_6$]PteGlu, free acid form |



## Pteroic acid

| | |
|---|---|
| $C_{14}H_{12}N_6O_3$<br>MW 312.28 | Pte, free acid form |

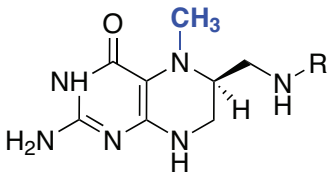


## 4-Aminobenzoylglutamic acid (PABGA)

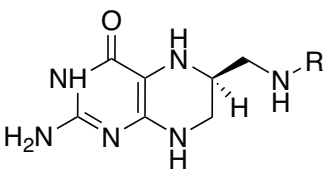
| | |
|---|---|
| $C_{12}H_{14}N_2O_5$<br>MW 266.25 | 7-(4-Aminobenzoyl)-L-glutamic acid,<br>free acid form |
| $C_6{}^{13}C_6H_{14}N_2O_5$<br>MW 272.25 | 7-(4-Amino[$^{13}C_6$]benzoyl)-L-glutamic<br>acid, free acid form |

Structure and nomenclature of natural diastereoisomer
*$^{13}C_5$-labeled form available
*$^{13}C_6$-labeled form available
*$^{13}C_7$-labeled form available

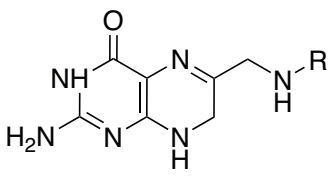
# Natural Folates



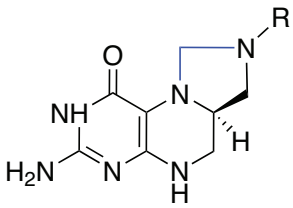
(6S)-**5-Methyl**tetrahydrofolate



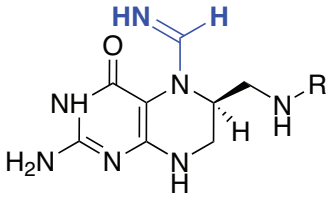
(6S)-Tetrahydrofolate



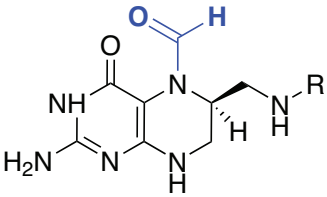
7,8-Dihydrofolate



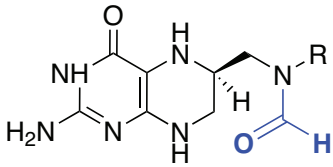
(6R)-**5,10-Methylene**tetrahydrofolate



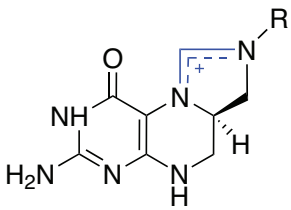
(6S)-**5-Formimino**tetrahydrofolate



(6S)-**5-Formyl**tetrahydrofolate

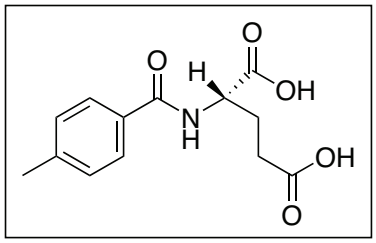


(6R)-**10-Formyl**tetrahydrofolate



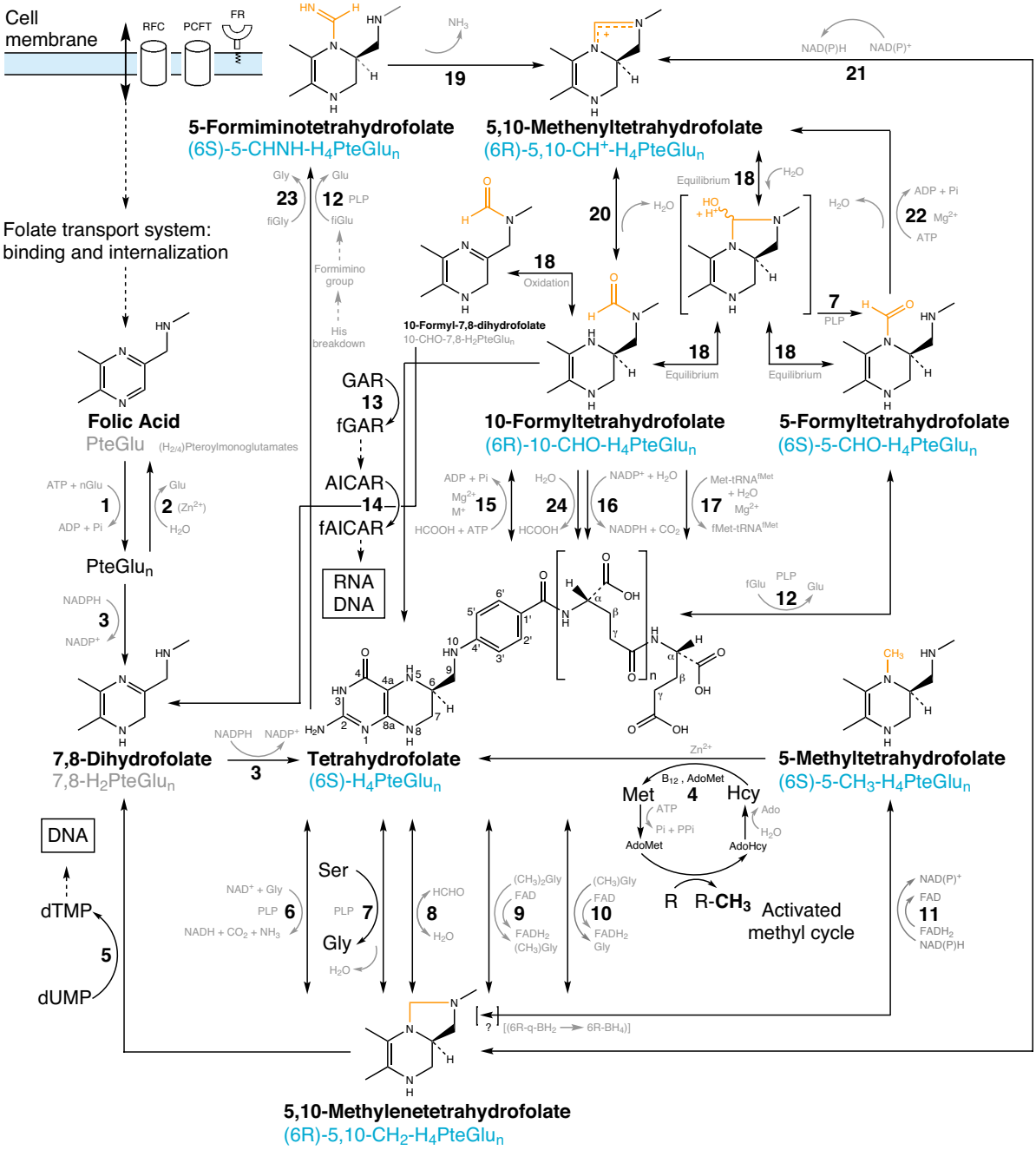
(6R)-**5,10-Methenyl**tetrahydrofolate

R =



benzoyl-*L*-glutamic acid

# Biochemical Pathways of Folates




1 Folylpolyglutamate synthase EC 6.3.2.17 (FPGS, FPGS)
2 γ-Glutamyl hydrolase EC 3.4.19.9
3 Dihydrofolate reductase EC 1.5.1.3 (DHFR, DHFR, DHFR2)
4 Methionine synthase EC 2.1.1.13 (MTR)
5 Thymidylate synthase EC 2.1.1.45 (TYMS, TYMS, TYMS)
6 Glycine cleavage system[1] (GLDC/DLD/AMT/GCSH)
7 Serine hydroxymethyltransferase EC 2.1.2.1 (SHMT1, SHMT2α, SHMT1, SHMT2α, SHMT2)
8 Equilibrium (non-enzymatic reaction)
9 Dimethylglycine dehydrogenase EC 1.5.99.2 (DMGD)
10 Sarcosine dehydrogenase EC 1.5.8.3 (SARDH)
11 5,10-Methylenetetrahydrofolate reductase EC 1.5.1.20 (MTHFR)
12 Formimidoyltransferase-cyclodeaminase EC 2.1.2.5[2] (FTCD)
13 Trifunctional purine biosynthetic protein adenosine-3 EC 2.1.2.2 (GART)
14 Bifunctional purine biosynthesis protein PURH EC 2.1.2.3 (ATIC)

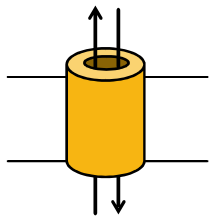
cytosolic
mitochondrial
nuclear

15 C-1-tetrahydrofolate synthase, EC 6.3.4.3[3] (MTHFD1, MTHFD1)
Monofunctional C1-tetrahydrofolate synthase EC 6.3.4.3 (MTHFD1L)
16 10-Formyltetrahydrofolate dehydrogenase EC 1.5.1.6 (ALDH1L1, ALDH1L2)
17 Methionyl-tRNA formyltransferase EC 2.1.2.9 (MTFMT)
18 Non-enzymatic reactions
19 Formimidoyltransferase-cyclodeaminase EC 4.3.1.4[2] (FTCD)
20 C-1-tetrahydrofolate synthase EC 3.5.4.9[3] (MTHFD1, MTHFD1)
Bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase EC 3.5.4.9[4] (MTHFD2/MTHFD2L)
21 C-1-tetrahydrofolate synthase EC 1.5.1.5[3] (MTHFD1, MTHFD1)
Bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase EC 1.5.1.15[4] (MTHFD2/MTHFD2L)
22 5-Formyltetrahydrofolate cyclo-ligase EC 6.3.3.2 (MTHFS)
23 Glycine formimidoyltransferase EC 2.1.2.4
24 10-Formyltetrahydrofolate deformylase EC 3.5.1.10

1) Glycine cleavage system (EC 1.4.4.2, EC 1.8.1.4, EC 2.1.2.10)
2) Bifunctional enzyme in eukaryotes (EC 2.1.2.5, EC 4.3.1.4)
3) Trifunctional enzyme in eukaryotes (EC 6.3.4.3, EC 1.5.1.5, EC 3.5.4.9)
4) Bifunctional enzyme in eukaryotes (EC 1.5.1.15, EC 3.5.4.9)

# Cellular Folate Transport Systems

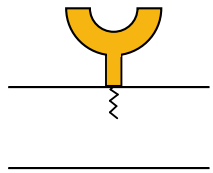
## Reduced Folate Carrier (RFC)



- Organic anion antiporter
- Major folate transport system

| | |
|---|---|
| Type: | Integral membrane glycoprotein, 12 transmembrane domains |
| pH Optimum: | 7.4 |
| Tissues: | Placenta, liver, leukocytes, kidney, lung, bone marrow, intestine, CNS, brain |
| Affinities: | (6S)-5-$CH_3$-$H_4$PteGlu (~ 5 µM) >> PteGlu (~ 200 µM) |
| Synonym: | Solute carrier family 19 member 1 |

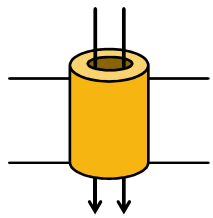
## Folate Receptor (FR)



- Transport via receptor mediated endocytosis
- Expressed on the cell surface

| | |
|---|---|
| Type: | Anchored to cell surface by a glycosylphosphatidylinositol (GPI) domain |
| Isoforms: | FR-α, FR-β, FR-γ (secreted), FR-δ |
| Tissues: | FR-α: Epithelial tissues (e.g. placenta, proximal renal tubular cells, choroid plexus)<br>FR-α levels are greatly elevated in malignant tissues<br>FR-β: Hematopoietic tissues (e.g. spleen, thymus, bone marrow), macrophages, fetal brain<br>FR-γ: Hematopoietic tissues (e.g. spleen, thymus, bone marrow)<br>FR-δ: Regulatory T-cells, Oocytes |
| Affinities: | (6S)-5-$CH_3$-$H_4$PteGlu (≥ 1 nM) < PteGlu (< 1 nM) |
| Synonym: | Folate binding protein (FBP) |

## Proton-Coupled Folate Transporter (PCFT)



- Proton symporter
- Major transport system for intestinal folate absorption

| | |
|---|---|
| Type: | Integral membrane glycoprotein, 12 transmembrane domains |
| pH Optimum: | 5.5 |
| Tissues: | Intestine, kidney, liver, placenta, spleen, brain, testis, lung |
| Affinities: | (6S)-5-$CH_3$-$H_4$PteGlu (~ 1 µM) = PteGlu (~ 1 µM) |
| Synonym: | Heme carrier protein 1 (HCP1), solute carrier family 46 member 1 |

## ATP-Binding Cassette (ABC) Transporters

- ATP dependent translocation of a wide variety of substances across membranes
- Export of folates (e.g. MRP1-5 and BCRP)

| | |
|---|---|
| Type: | Transmembrane protein |
| Affinities: | Low-affinity, high-capacity pumps |

# Folate History

**1931** Lucy Wills discovers that macrocytic anaemia can be prevented by adding yeast to a diet otherwise lacking B vitamins

**1941** Herschel K. Mitchell and colleagues suggest the name “Folic acid” (folium, Latin for leaf) for the factor responsible for growth stimulation of Streptococcus lactis isolated from spinach

**1945** Tom D. Spies demonstrates that Folic acid cures megaloblastic anaemia during pregnancy

**1946** Robert B. Angier and co-workers report the structure and synthesis of the Lactobacillus casei factor (Folic acid) isolated from liver

**1950** Emanuel B. Schoenbach and colleagues observe that the toxic side effects of amethopterin (methotrexate) cancer therapy can be reversed by treatment with “citrovorum factor” (Leucovorin)

**1962** Victor Herbert consumes a folate-deficient diet for several months, documenting the development of deficiency symptoms

**1968** Martin C. Carey and colleagues report that oral Folic acid therapy significantly reduces homocysteine excretion in the urine of mentally-handicapped children with homocystinuria

**1981** Richard W. Smithells and co-workers report a preventive effect on neural tube defects by a periconceptional vitamin supplementation containing Folic acid

**1982** David Machover and colleagues demonstrate that (6R,S)-5-Formyltetrahydrofolate or “Folinic acid” (Leucovorin) increases the therapeutic efficacy of 5-fluorouracil in the treatment of advanced colorectal and gastritic adenocarcinomas

**1991** Nicholas Wald demonstrates in a randomised double-blind prevention trial that Folic acid supplementation before pregnancy reduces the risk of neural tube defects by 70% in women who had previously given birth to a child with a neural tube defect

**1991** Eprova AG succeeds in producing (6S)-5-Formyltetrahydrofolate (Levoleucovorin), the natural isomer of (6R,S)-5-Formyltetrahydrofolate (Leucovorin), in commercial quantities

**1992** Andrew E. Czeizel finds that first occurrence of neural tube defects may be prevented by periconceptional Folic acid supplementation

**1992** The U.S. Public Health Service recommends women of childbearing age to consume 0.4 mg of Folic acid daily

**1997** Mary Ward and colleagues demonstrate that plasma homocysteine can be lowered by physiological doses of Folic acid

**1998** The U.S. Food and Drug Administration (FDA) introduces mandatory fortification of flour, rice, pasta, and other grain products with Folic acid

**2001** The U.S. Food and Drug Administration (FDA) accepts (6S)-5-Methyltetrahydrofolate (Metafolin®) for use in dietary supplements

**2004** The European Food Safety Authority (EFSA) considers (6S)-5-Methyltetrahydrofolate (Metafolin®) safe

**2005** The “Joint FAO/WHO Expert Committee on Food Additives” (JECFA) considers (6S)-5-Methyltetrahydrofolate (Metafolin®) safe

**2008** The U.S. Food and Drug Administration (FDA) approves (6S)-5-Formyltetrahydrofolate (Fusilev®) for rescue after high-dose methotrexate therapy

**2010** The U.S. Food and Drug Administration (FDA) approves Beyaz® a combination oral contraceptive containing (6S)-5-Methyltetrahydrofolate (Metafolin®) to prevent neural tube defects

**2015** The Center for Radiopharmaceutical Sciences (CRS), a joint endeavor between the Paul Scherrer Institute, the ETH Zurich and the University Hospital Zurich starts the first-in-man clinical trial with a novel folate-based [$^{18}$F]-PET tracer for imaging of folate receptor-positive tumors

**2018** Isofol Medical AB reports positive efficacy data for (6R)-5,10-Methylenetetrahydrofolate (Modufolin®) from patients treated for metastatic colorectal cancer

SAFC®

Pharma & Biopharma Raw Material Solutions

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany

**MerckMillipore.com**

The typical technical data above serve to generally characterize the product. These values are not meant as specifications and they do not have binding character. To learn more about the product specifications and to place an order, please send an e-mail to **folates@merckgroup.com**

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect of any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

**For additional information, please visit MerckMillipore.com**
**To place an order or receive technical assistance, please visit MerckMillipore.com/contactPS**

**For more information please contact:**
Phone: +41 52 630 72 72
E-mail: folates@merckgroup.com



Lit. No. MK_BR2972EN Ver. 1.0
2018-11753 05/2018