

This article was downloaded by:

On: 27 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597286>

Chemical Synthesis and Cloning of a Gene Fragment Designed to Aid Polypeptide Purification

L. D. Bell^a; J. C. Smith^a; R. B. Derbyshire^a; E. Cook^a; L. Dunthorne^a; J. Viney^a; S. Brewer^a; H. Sassenfeld^a

^a Searle Research and Development, Buckinghamshire, U.K.

To cite this Article Bell, L. D. , Smith, J. C. , Derbyshire, R. B. , Cook, E. , Dunthorne, L. , Viney, J. , Brewer, S. and Sassenfeld, H.(1985) 'Chemical Synthesis and Cloning of a Gene Fragment Designed to Aid Polypeptide Purification', *Nucleosides, Nucleotides and Nucleic Acids*, 4: 1, 307

To link to this Article: DOI: 10.1080/07328318508077902

URL: <http://dx.doi.org/10.1080/07328318508077902>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

CHEMICAL SYNTHESIS AND CLONING OF A GENE FRAGMENT DESIGNED TO AID
POLYPEPTIDE PURIFICATION

L.D.Bell*, J.C.Smith, R.B.Derbyshire, E.Cook, L.Dunthorne,
J.Viney, S.Brewer, H.Sassenfeld.
Searle Research and Development, PO Box 53, Lane End Road,
High Wycombe, Buckinghamshire, U.K.

Summary: The design, synthesis and cloning of a 43 bp DNA duplex coding for polyarginine is described. It has been used to modify the isoelectric point of human urogastrone and thereby facilitate purification by ion-exchange chromatography.

Purification of genetically engineered proteins from a bacterial lysate can be difficult on a large scale. Ion exchange chromatography is particularly suited to large scale purification but is unlikely to resolve polypeptides of similar overall charge. We decided to investigate a general method by which the isoelectric point of any protein could be modified. As a model system we chose urogastrone for which we have previously synthesised a gene (1).

A polyarginine C-terminal tail of 6 residues was chosen since this was shown to have minimal effect on biological activity, it could readily be removed with carboxypeptidase B and significantly increases the pI of urogastrone. The gene sequence was carefully designed to avoid potential problems related to tRNA limitation and secondary structure in the mRNA.

We have shown that cells containing polyarginine fused urogastrone grow at a similar rate to those containing only urogastrone. In addition the polyarginine tail confers an increased resistance to endogenous proteases.

Ion exchange HPLC data have shown the significant advantage of this general method for polypeptide purification.

REFERENCES

- (1) Smith *et al.*, Nucleic Acids Research. (1982), **10**, 4467-4481.