Tetrahedron Letters, Vol.31, No.24, pp 3493-3494, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

SYNTHESIS OF THE N-GLYCOPEPTIDE PARTIAL SEQUENCE A^1-A^{12} OF THE β-CHAIN OF GLYCOSYLATED HAEMOGLOBIN HbA_{1C}. A NEW APPROACH TO AMADORI N-GLYCOPEPTIDES.

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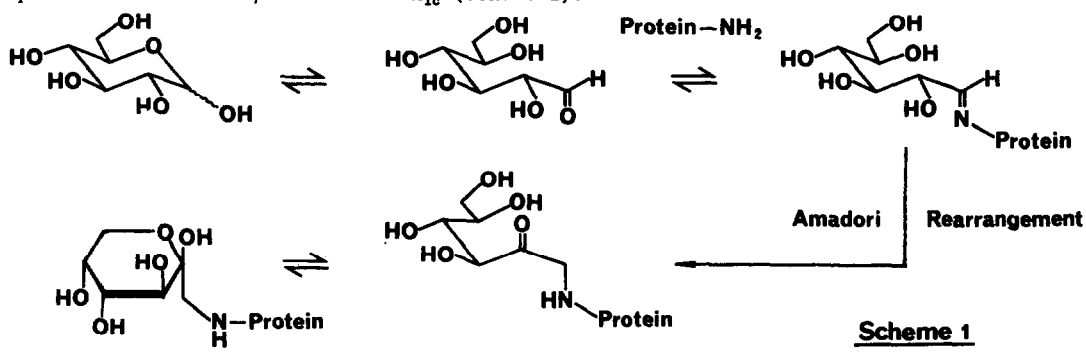
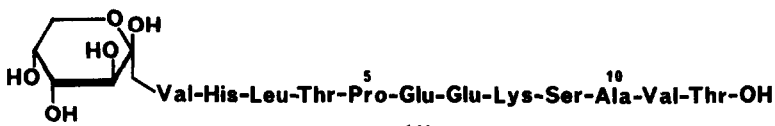
Abstract: A new approach to a terminal partial sequence of the β-chain of HbA_{1c} is described whereby a protected Amadori amino acid is incorporated into a solution-phase peptide synthesis.

Glycoproteins play an important role in biological processes and as such are the subject of much current research interest in an effort to develop new and selective methodology towards their synthesis[2]. Normal adults have about 10% of their total haemoglobin glycosylated of which about 5% consists of the HbA_{1c} form in which a glucose moiety is attached to the amino group of the N-terminal valine of the β-chain[3]. Extensive study by a number of workers[4] has established that glucose reacts initially with the N-terminal amino group to form a labile Schiff base aldimine which subsequently undergoes irreversible Amadori rearrangement followed by reversible cyclisation to form a 1-amino-1-deoxy-fructopyranose derivative of structure (1) for the partial sequence A^1-A^{12} of the β-chain of HbA_{1c} (Scheme 1).



Scheme 1

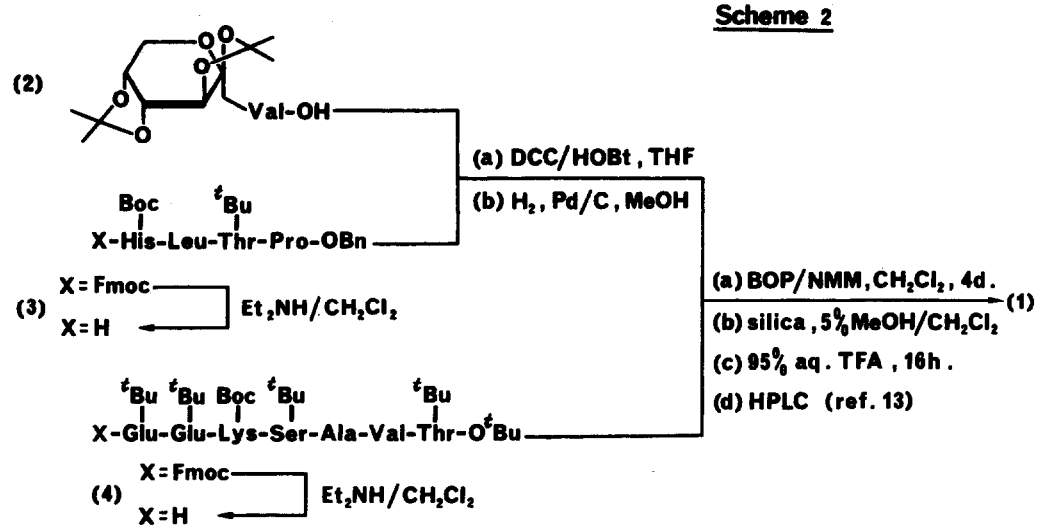
We required a highly pure sample of (1) for the screening of antibodies against HbA_{1c}. Previous synthetic efforts in this field have involved simply incubating glucose (or maltose) with an unprotected peptide in phosphate buffer[5,6] or pyridine/acetic acid[6-8] on the assumption that the Amadori product is obtained. Our reservations with this method were that the yield would be low due to the formation of complex mixtures of products derived from the non-enzymatic 'browning' or Maillard reaction[9] of the initial Amadori compound and that glycosylation may not be selective in that reaction could occur to some extent at the ϵ-amino group of lysine-8.



(1)

We present here a new and unambiguous approach to (1) which involves synthesis of the protected valine Amadori compound (2) and its incorporation into a solution-phase peptide synthesis thus avoiding the possibility of side-reactions and non-selectivity mentioned above for direct glycosylation. Our overall strategy (see Scheme 2) was to construct the dodecapeptide from two fragments by coupling at the proline-5 residue which is less susceptible to racemisation. Acid labile side-chain protecting groups (Boc, t-Bu, isopropylidene) were used throughout to allow a single deprotection step.

Scheme 2



The heptapeptide (4) comprising the sequence A^6-A^{12} was synthesised in a stepwise manner using N-Fmoc protected amino acid pentafluorophenyl active esters starting from (O-^{t}Bu)Thr-O^{t}Bu[10] in 51% overall yield. Successive coupling products were purified by column chromatography on silica gel (ether/EtOAc mixtures) and identified by nmr spectroscopy. An attempt to carry out a similar strategy for the synthesis of (3) from Pro-OBn was unsuccessful when N-deprotection of Fmoc-(O-^{t}Bu)Thr-Pro-OBn afforded a diketopiperazine in high yield. Instead, we coupled Fmoc-Leu-(O-^{t}Bu)Thr-OH to Pro-OBn using DCC/HOBt followed by treatment of the resulting tripeptide with Et_2NH then Fmoc-(Boc)His-OPfp to provide the desired tetrapeptide (3) in 41% overall yield. The Amadori compound (2) was prepared as its methyl ester using a literature procedure[11]. Saponification (NaOH, MeOH) then led to the free acid (2) in 60% yield after column chromatography on silica gel (20% MeOH in EtOAc). Compound (2) was coupled to N-deprotected (3) using DCC/HOBt to afford a 40% yield of the protected Amadori glycopentapeptide. Hydrogenolysis of the C-terminal benzyl ester allowed the pentapeptide to be coupled to the N-deprotected heptapeptide (4) using the BOP reagent[12] to produce the fully protected form of (1). Treatment of the purified protected glycododecapeptide with 95% aq. TFA yielded (1) with a purity of 67% as indicated by HPLC. The crude peptide was subjected to preparative HPLC purification and characterised by FAB mass spectrometry (FABMS) and amino acid analysis[13].

Acknowledgement : We are grateful to Miss. V. Cleeter for typing this manuscript.

References and Notes.

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13. Amino acid analysis (molar ratio): Ala (1.07), Val (1.84), Thr (1.95), Ser (0.88), His (0.86), Leu (1.02), Glu (2.18), Lys (1.06), Pro (1.13); FABMS $[M + H]^+$ m/z 1472±1; HPLC retention time: 13.9 min (Vydac C_{18}, 4.6 x 250mm, H_2O - 0.1% TFA, linear gradient 0-50%CH_3CN - 0.1%TFA (30 min), flow rate 1.5ml/min).

(Received in UK 3 May 1990)