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Transient Affinity Tags Based on the Dde Protection/ Deprotection Strategy: Synthesis and Application of 2-Biotinyl- and 2-Hexanoyldimedone

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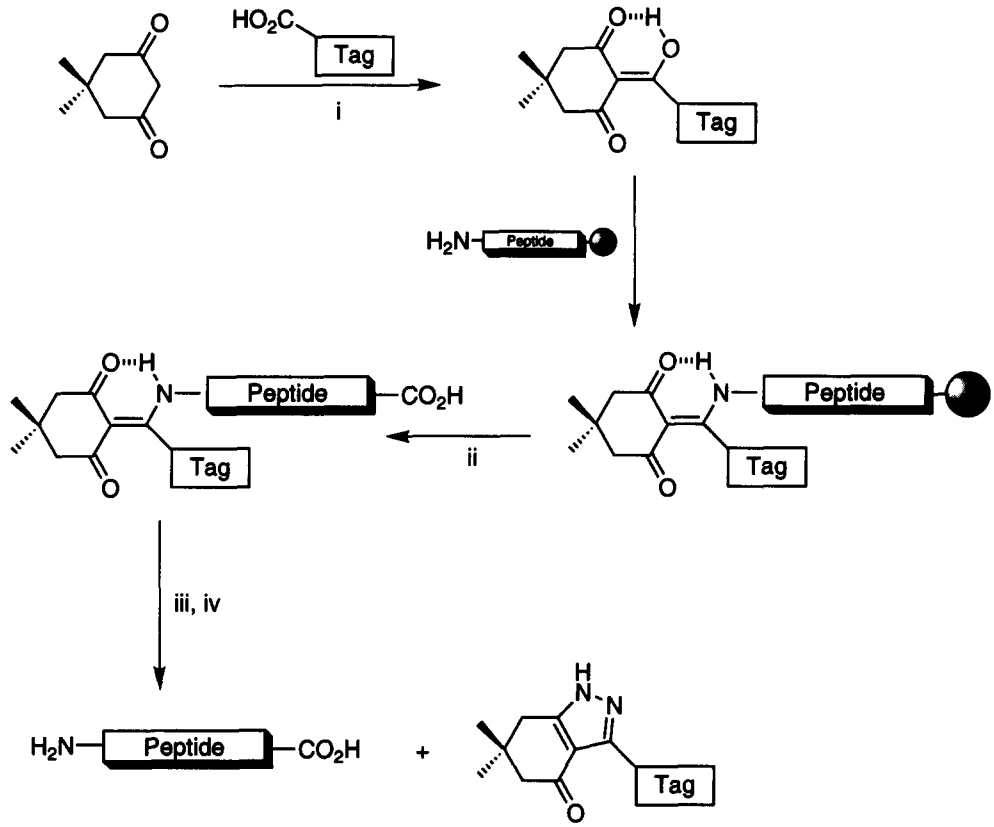
Abstract: 2-Biotinyl- and 2-hexanoyldimedone were prepared in a simple one step high yielding process by acylation of dimedone with biotin and hexanoic acid respectively, then without activation each attached to the *N*-terminus of resin-bound peptides. Following acidolytic side-chain deprotection and concomitant cleavage of the tagged peptides from the support, affinity purification was achieved on an avidin-agarose column and by RP-HPLC respectively. The purified peptides were finally released from the tag with 5% aqueous hydrazine.

The applications of affinity tagging continue to grow not only in relation to chromatographic methods[1], but also as a means of immobilising molecules onto defined surfaces for biophysical studies. Surface Plasmon Resonance (SPR)[2] and Scanning Force Microscopy (SFM)[3] are examples where the latter has particular significance. However the separation problems, particularly those associated with deletion peptides generated by solid phase synthetic methods when applied to large polypeptides, as well as short 'difficult sequence' peptides have represented the main focus to date[4].

A number of approaches have been adopted, the most common of which exploits the high affinity of avidin towards a biotinylated end-product. The free *N*-terminus of the peptide-resin assembled by a coupling and capping procedure can be functionalised with activated biotin[5]. However this procedure has the disadvantage that the biotin cannot subsequently be removed. Therefore reagents which afford a transient biotin tag based on the fluorenylmethoxycarbonyl (Fmoc) group[6,7] and the 2-(*N*-biotinylaminoethylsulphonyl)ethoxycarbonyl group[8] have been developed. These are removed by base and their general efficacy has been demonstrated. A range of other tagging strategies based on hydrophobic interactions have been described, these include tetrabenzo[*a,c,g,i*]fluorenyl-17-methylurethane (Tbfmoc) which displays a particularly strong affinity for porous graphitised carbon[9] and more recently an alternative application of the aminoethylsulphonylethoxycarbonyl group[10]. However, a number of the above procedures suffer from protracted syntheses of the reagents as well as a need to pre-activate before derivatisation. Our objective was to adapt the amine group protection/deprotection strategy based on the 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl group (Dde)[11] into a simple and generic tagging procedure. The approach we have adopted for the purification of synthetic solid phase peptides is outlined in **Scheme 1**.



Scheme 1

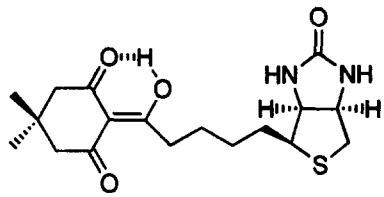
Reagents and conditions: i) DCC/DMAP; ii) TFA; iii) affinity column; iv) 5% hydrazine$_{(aq)}$

Without pre-activation, 2-acyldimedones can be reacted with a free primary amine group on any target molecule to give derivatives which are stable to both the acid and base conditions employed in solid phase peptide chemistry. The parent molecule is then released from the tag by treatment with aqueous hydrazine as and when required. In principle any tagging reagent with a free carboxylic acid group can be used to acylate dimedone in the manner shown. Using the synthesis of the eight residue angiotensin II receptor binding protein fragment[12] (**1**) to illustrate and validate the approach, we now report two examples of affinity attachments derived from dimedone and their applications.

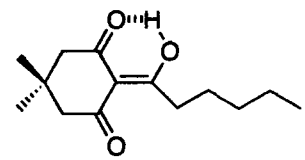
Crystalline 2-biotinyldimedone (2) was synthesised in good yield by reacting dimedone with biotin in the presence of DCC/DMAP at room temperature in DMF[13]. The spectral data was consistent with a *C*-acylated product and the ^{1}H nmr displayed the expected low field singlet at δ 14.85 characteristic of the strongly hydrogen bonded enolic proton. The peptide (1) was assembled using standard Fmoc solid phase peptide chemistry on a NovaSyn® KR 125 resin, and while still attached to the resin incubated overnight with a four fold excess of (2). The RP-HPLC of the product from TFA mediated cleavage and side-chain deprotection contained one major peak, the ES-MS of which exhibited the correct molecular ion for the biotinylated peptide. This product was then dissolved in phosphate buffered saline, adsorbed onto an avidin-agarose column, and washed with the same buffer to remove truncated peptides and impurities. RP-HPLC of the washings indicated complete loss of the biotinylated peptide peak confirming its adsorption on the column. To release pure (1) from the biotin-peptide conjugate, the column was eluted with 5% aqueous hydrazine.

H-Lys-Gly-Val-Tyr-Ile-His-Ala-Leu-NH_2

1



2



3

2-Hexanoyldimedone (3) was prepared[14] and used to derivatise the terminal amino group of resin-attached peptide (1) in an analogous manner to (2). RP-HPLC of the *N*-acylated peptide following TFA treatment revealed one major peak at 16.8 min, compared with 7.5 min for the free peptide (1)[15]. This significant shift in retention time was sufficient to allow preparative separation from impurities using RP-HPLC. Subsequent removal of the hydrophobic tag with 5% aqueous hydrazine[16] afforded pure (1) virtually indistinguishable from the material obtained using the biotinylating agent (2).

We are currently evaluating both procedures with respect to large synthetic peptides, PNAs and other solid phase constructs. In addition we are considering other attachments to Dde such as histidine tags.

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References and Notes

Abbreviations: For amino acids and peptides follow the IUPAC-IUB nomenclature where applicable (Eur. J. Biochem., **1984**, 9-37); DCC, *N,N'*-dicyclohexylcarbodimide; DMAP, 4-dimethylaminopyridine; DMF, *N,N'*-dimethylformamide; ES-MS, electrospray mass spectrometry; Fmoc, 9-fluorenylmethoxycarbonyl; PNAs, peptide nucleic acids; RP-HPLC, reversed phase high performance liquid chromatography; TFA, trifluoroacetic acid

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13. **2-Biotinyldimedone (2):** To a stirred solution of biotin (0.244 g, 1.0 mmol) in DMF (10 ml), dimedone (0.154 g, 1.1 mmol), DCC (0.206 g, 1.0 mmol) and DMAP (0.122 g, 1.0 mmol) were added and the mixture allowed to stir at room temperature for 48 h. Precipitated DCU was removed, the solution evaporated and the residue redissolved in ethyl acetate. The organic solution was washed with 1M $KHSO_4$ solution and the product extracted with $NaHCO_{3\,(aq)}$. Extraction of the acidified aqueous solution with dichloromethane afforded, on evaporation and recrystallisation from methanol-water (1:1), **(2)** as a white crystalline solid (0.25 g, 69%), m.p. 156-157 °C

^{1}H NMR (250 MHz) δ 1.07 (6 H, s, $C(CH_3)_2$), 1.54 (6 H, m, $(CH_2)_3$), 2.35 (2 H, s, $COCH_2$), 2.54 (2 H, s, $COCH_2$), 2.73 (1 H, d, *J* 13 Hz, $CHSCH_\beta$), 2.91 (1 H, dd, *J* 13, 5 Hz, $CHSCH_\alpha$), 3.04 (2 H, t, *J* 7 Hz, $=C(OH)CH_2$), 3.16 (1 H, m, $CHSCH_2$), 4.31 (1H, m, ring junction H), 4.56 (1H, m, ring junction H), 5.87 (1H, s, NH), 6.15 (1H, s, NH), 14.85 (1H, s, OH). ^{13}C NMR δ 24.26 ($C(OH)CH_2CH_2$), 28.02 ($C(CH_3)_2$), 28.19 ($C(OH)CH_2CH_2CH_2CH_2$), 28.45 ($C(OH)CH_2CH_2CH_2$), 30.50 ($C(CH_3)_2$), 39.87 ($C(OH)CH_2$), 40.44 (SCH_2), 46.60 (CH_2CO), 52.44 (CH_2CO), 55.47 ($SCHCH_2$), 60.00 (SCH_2CH), 61.86 (SCHCH), 111.80 ($C=C(OH)CH_2$), 164.01 (NHCONH), 195.01 (CH_2CO), 197.55 (CH_2CO), 205.191 ($=C(OH)CH_2$)

Found: C, 59.15; H, 7.07; N, 7.39. $C_{18}H_{27}N_2O_4S$ requires C, 59.00; H, 7.15; N, 7.65%

HRMS (FAB) Found: *m/z* 367.162687. Calcd. for $C_{18}H_{27}N_2O_4S$ (M+H), 367.169154.
14. **2-Hexanoyldimedone (3):** Pale yellow oil; ^{1}H NMR (90 MHz) δ 0.85 (3 H, t, *J* 6 Hz, CH_2CH_3), 1.05 (6 H, s, $C(CH_3)_2$), 1.40 (6 H, m, $(CH_2)_3CH_3$), 2.30 (2 H, s, $COCH_2$), 2.47 (2H, s, $COCH_2$), 2.93 (2 H, t, *J* 7 Hz, $=C(OH)CH_2$), 18.14 (1H, s, enol OH) *m/z* (+ve ES-MS) 239.1 (M+H), calcd. for $C_{14}H_{23}O_3$ (M+H), 239.
15. Hypersil Pep C_{18} analytical column (4.6 x 150 nm); mobile phase, A: 0.06% TFA aq, B: 0.06% TFA in 90% MeCN/H_2O, linear gradient 10% to 50% B in 20 min at 1.2 ml/min and post column eluent detection at 220 nm.
16. A solution of the *N*-terminal tagged peptide (3.0 mg) in 5% of aqueous hydrazine (3 ml) was stirred at room temperature for 30 min. The solution was acidified with TFA and desalted using a Millipore C_{18} Sep-Pak cartridge. Evaporation of the desalted solution gave after ether trituration the pure peptide as a white amorphous solid (2 mg).

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