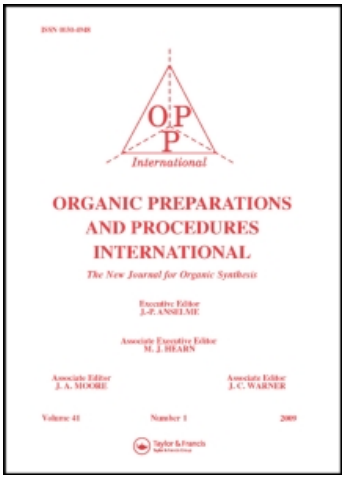

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A FACILE SYNTHESIS OF BIOTIN N-HYDROXYSUCCINIMIDE ESTER

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A FACILE SYNTHESIS OF BIOTIN N-HYDROXYSUCCINIMIDE ESTER

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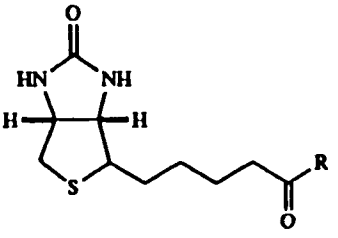
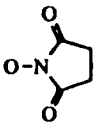
The exceptionally high affinity ($K_d 10^{-15} M$) of avidin, a tetrameric egg white protein of M_r 67,000, for biotin (1) to form the avidin-biotin noncovalent complex has been exploited successfully for the isolation of hormone receptors, affinity chromatography, immunoassay, localization and detection of proteins and enzymes by the blot transfer techniques as evidenced by the growth of avidin-biotin technology.[1] Since only the bicyclic ring of the biotin molecule is important for binding to avidin,[2] the carboxyl group of the valeric acid side-chain has been chemically modified and functionalized to give suitable biotin derivatives for coupling to low- and high-molecular weight molecules.[1] Of the various biotinylating reagents, the active esters of biotin, the p-nitrophenyl ester and the N-hydroxysuccinimide ester, are most commonly used. The synthesis of biotin p-nitrophenyl ester[3] is not straightforward and side-reactions via participation of the ureido nitrogen of the biotin ring in N-acylation and isouronium salt formation have been reported.[3] In view of these difficulties, Hofmann et al.[4] chose the N-hydroxysuccinimide ester of biotin in their work. Since the dicyclohexylcarbodimide (DCC) procedure for the synthesis of N-hydroxysuccinimide esters of N-protected amino acids[5] gave an impure product with biotin,[6,7] a better method employing N,N'-carbonyldiimidazole (CDI) was reported by Jasiewicz et al.[7] The use of DCC reagent leads, besides dicyclohexyl urea as a

contaminating side-product, to side-reactions[3] through the participation of the ureido nitrogen of the biotin ring.



1. R = -OH

2. R = 

3. R = $-NH_2$

We have found yet another method for the synthesis of biotin N-hydroxysuccinimide ester (2) using N,N'-disuccinimidyl carbonate[8] (DSC) as the condensing agent. This reagent has been found to be less moisture sensitive than CDI and does not give side-reactions of the previous methods. Using equimolar amounts of the reagent DSC, biotin and dry pyridine (or N-methylmorpholine) as base, the reaction carried out in DMF as solvent gave 83% yield (59% overall yield after recrystallization) of the desired active ester. Instead of the ususal recrystallization of the active ester from hot 2-propanol which leads to decomposition and lower recovery, we found it is preferable to reprecipitate it from DMF-2-propanol to give pure biotin N-hydroxysuccinimide ester as evidenced by TLC, melting point and NMR spectrum. Derivatization of the active ester by reaction with methanolic ammonia gave the known biotinamide (3)[10] in 84% yield. The procedure described here is convenient and, after minimal work-up, gives a high yield of biotin N-hydroxysuccinimide ester (2).

EXPERIMENTAL SECTION

Mps. were determined with a Buchi apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on Whatman K6F-silica gel glass plates (0.25 mm) using the following solvent systems (v/v/v): n-butanol-glacial acetic acid-water, 3/1/1, (A); chloroform-methanol, 11/7, (B); n-butanol-glacial acetic acid-water, 15/6/5, (C). Visualization was effected with ultraviolet light, iodine vapor and 0.2% solution of p-dimethylaminocinnamaldehyde[9] reagent in ethanol containing 2% sulfuric acid. The NMR spectra were recorded on a Varian 390 or XL-400 spectrometer. Chemical shifts are reported as parts per million (δ) relative to tetramethylsilane as the internal standard.

Biotin N-Hydroxysuccinimide Ester (2).- To a stirred solution of (+)biotin (0.732 g, 3.0 mmol) in 9 mL of dry DMF held at 60° were added anhydrous pyridine (0.3 mL, 3.5 mmol) and N,N'-disuccinimidyl carbonate (Aldrich, 0.8 g, 3.13 mmol). The reaction mixture was stirred at 60° for 3 hrs and then at room temperature for 2 hrs. Solvents were removed under reduced pressure and the residue was stirred with 2-propanol (15 mL) and cooled to 0°. The precipitate was collected, washed with 2-propanol, ether and dried in vacuo to give 0.85 g (83%) of crude product, mp. 180-185°. It was reprecipitated by dissolving in 10 mL of DMF at 40° and adding 40 mL of 2-propanol to give 0.6 g (59%) of biotin N-hydroxysuccinimide ester as white crystals, mp. 205-208°. A small portion was recrystallized from 2-propanol to give a pure sample, mp. 210°, lit.[6,7] 196-200°; TLC homogeneous R_f 0.76 (A), 0.81 (B), 0.51 (C);

$[\alpha]_D^{21}$ + 59° (c 2, DMF); ^{1}H NMR (Me_2SO-d_6, 90 MHz): δ 1.25-1.85 (6H, m, -$(CH_2)_3$-side-chain of biotin), 2.43-2.9 (8H, m, $\underline{CH_2}S$ biotin ring + m of $\underline{CH_2}CO$ of side-chain + $(CH_2)_2$ of succinimido, the latter centered at 2.8), 3.05-3.25 (1H, m, $\underline{CH}S$ of biotin ring), 4.1-4.45 (2H, m, NH$\underline{CHCH}$NH of bicyclic bridge of biotin ring), 6.42, 6.35 (2H, closely spaced singlets, $\underline{NH}CO\underline{NH}$ of biotin ring).

Anal. Calcd. for $C_{14}H_{19}N_3O_5S$: C, 49.25; H, 5.61; N, 12.31

Found : C, 48.84; H, 5.56; N, 11.95

The above active ester was treated with conc. ammonium hydroxide in anhydrous methanol at room temperature overnight to give biotinamide (3) in 84% yield, mp. 244-246°, lit.[10] 243-244°; TLC homogeneous R_f 0.6 (A), 0.71 (B). ^{1}H NMR (Me_2SO-d_6, 400 MHz): δ 1.3-1.7 (6H, m, -$(CH_2)_3$-side-chain), 2.03 (2H, t, J = 7.2 Hz, -$\underline{CH_2}CONH_2$), 2.58 and 2.82 (1H each, d, J = 12.6 Hz and dd, J = 12.6 and 5.0 Hz, respectively, $\underline{CH_2}S$ of biotin ring), 3.1 (1H, m, $\underline{CH}S$ of biotin ring), 4.15 and 4.30 (1H each, m, -NH$\underline{CHCH}$NH- of bicyclic bridge of biotin ring), 6.36 and 6.43 (1H each, s, ureido NH of biotin ring), 6.69 and 7.23 (1H each, br s, $CO\underline{NH_2}$).

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