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SYNTHESIS OF FLUORESCENT AND BIOTINYLATED ANALOGUES OF (1*R*, 2*S*, 3*R*)-2-ACETYL-4(5)-(1,2,3,4-TETRAHYDROXYBUTYL)IMIDAZOLE

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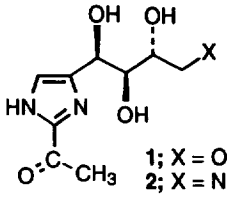
Abstract: **A method for preparing fluorescent and biotinylated analogues of the biologically active compound (1*R*, 2*S*, 3*R*)-2-acetyl-4(5)-(1,2,3,4-tetrahydroxybutyl)imidazole 1 is reported.**

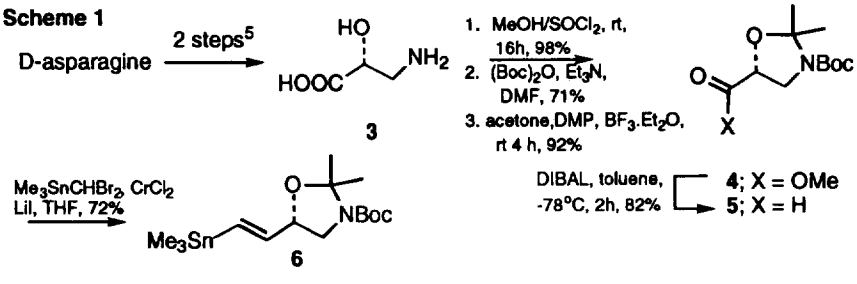
(1*R*, 2*S*, 3*R*)-2-Acetyl-4(5)-(1,2,3,4-tetrahydroxybutyl)imidazole (THI) **1**, a constituent of Caramel Colour III, has been found to depress blood lymphocyte counts in both mice and rats.[1] THI produces lymphopenia, apparently without toxic effects, in rats and mice and is able to affect the immune competence in the rat in quite small quantities (e.g. 1-50 ppm in drinking water).[2] THI has also been reported to prevent spontaneous and cyclophosphamide-induced diabetes in non-obese diabetic mice.[3]



1; X = OH
2; X = NH_2

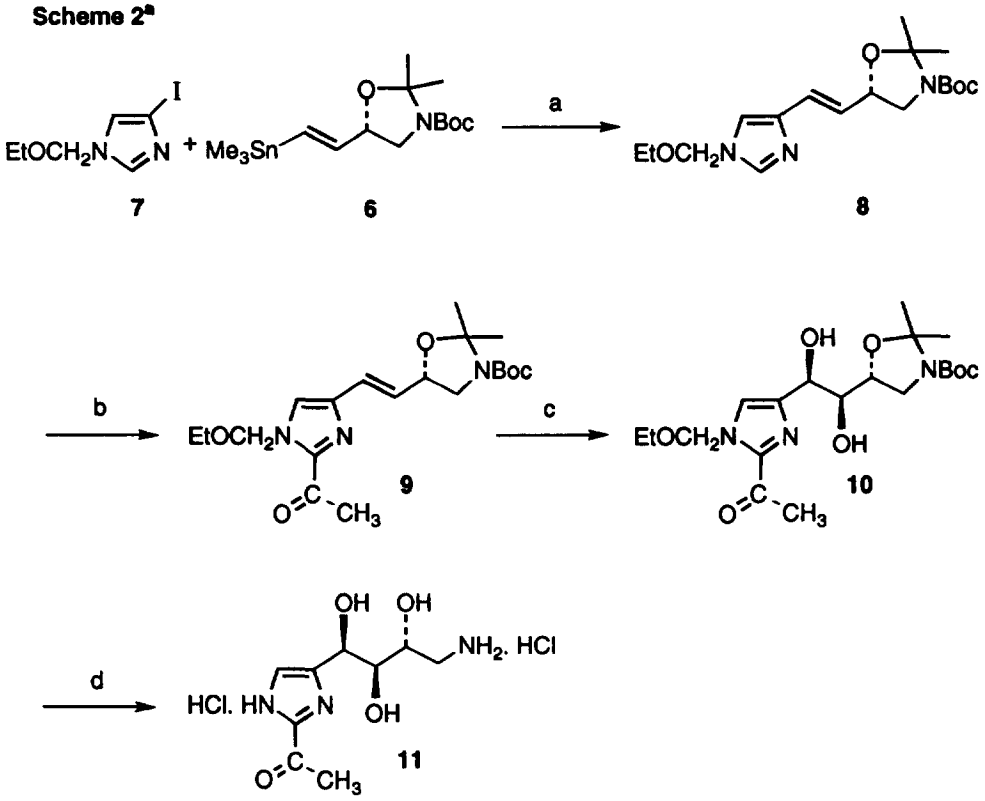
To investigate the binding of THI to T-cells or other biological receptors we required a synthesis of a fluorescent or biotinylated derivative of THI. We report here the synthesis of the hydrochloride salt of (1*R*, 2*S*, 3*R*)-2-acetyl-4(5)-(1,2,3,-trihydroxy-4-amino-butyl)imidazole **2** using a modification of our recently reported synthesis of THI[4] and the preparation of its dansyl, fluorescein and biotin derivatives for cell and receptor binding studies. The synthesis of **2** involved a palladium catalyzed coupling of the 1-protected-4-iodoimidazole **7** to the functionalized vinylstannane **6** to produce the (*E*)-alkene **8** and the Sharpless catalytic asymmetric dihydroxylation (AD) to introduce the (1*R*, 2*S*)-dihydroxy functionality into the butyl side chain of **2** (Scheme 2). The vinylstanne **6** was prepared from D-asparagine according to Scheme 1. D-asparagine was converted to (*R*)-isoserine **3** using the literature procedures for the synthesis of the (*S*)-enantiomer of **3** from L-asparagine.[5] (*R*)-isoserine **3** was then converted to its methyl ester with thionyl chloride/methanol

Scheme 1



(RT, 16 h, 98% yield) which was converted to the *N*-Boc-oxazolidine **4**[6] using the procedures developed by McKillop and Taylor[7] for the synthesis of the related 4-methoxycarbonyl-*N*-Boc-oxazolidine that was first reported by Garner.[8] Reduction of **4** with DIBAL[8] gave the aldehyde **5**[6] in 82% yield after bulb-to bulb distillation (bp 85-90°C / 2 mm Hg). The aldehyde **5** was smoothly converted to the vinylstanne **6**[6] ((*E*) : (*Z*) = 75 : 25) in 72% yield using our previously disclosed procedure employing trimethyldibromomethylstanne and chromium(II) chloride/ lithium iodide in THF.[9]

Scheme 2[a]



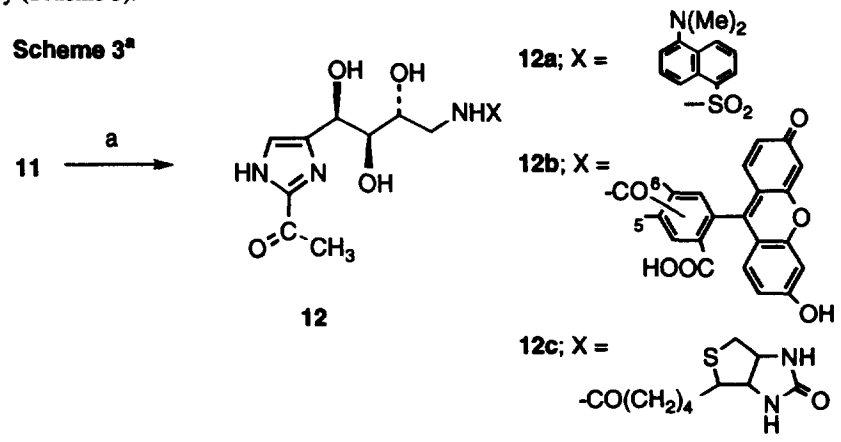
[a]Key: (a) 5% $Pd(PPh_3)_4$, DMF, 80 °C, 24 h, 34 %; (b) (i) *n*-BuLi, THF, -78 °C, 1h, (ii) MeCONMe(OMe), -78 °C (1 h) to rt (1 h), 64 %; (c) AD mix-β, $(DHQD)_2$-PHAL (4 mol %) , methanesulfonamide (2 equiv.), *t*-BuOH / H_2O, 0 °C, 4 days, 81 %; (d) 10% HCl / ethanol (2 : 1),80 °C 1.75 h, 100%.

A Stille type coupling reaction[4,10-12] of 1-ethoxymethyl-4-iodoimidazole **7**[12] and vinylstannane **6** using 5 % $Pd(PPh_3)_4$ in DMF at 80 °C for 24 h, gave the pure (*E*)-alkene **8**[6] in 34 % yield after purification by column chromatography to remove the small amount of the isomeric (*Z*)-alkene product. Treatment of **8** with *n*-butyllithium (1.2 equiv.) in THF at -78 °C for 1 h, followed by quenching the resulting 2-lithio-imidazole derivative with *N*-methoxy-*N*-methyl acetamide[12,13] (1.4 equiv., -78 °C (1 h) to rt (1 h)) gave the 2-acetylimidazole **9**[6] in 64 % yield based on recovered starting material (19% recovered). Catalytic asymmetric dihydroxylation (AD) of **9** at 0 °C for 4 days using commercially available AD mix-β,[13,14] additional chiral

ligand ((DHQD)$_2$-PHAL (4 mol %) and methanesulfonamide (2 equiv.) in *t*-BuOH / H_2O (1 : 1) gave the *syn* (1*R*, 2*S*)-diol **10**[6] in good yield (81 %) and high diastereoselectivity (d.e. >98 %) as determined by ^1H NMR analysis. Hydrolysis of **10** with aqueous 10 % hydrochloric acid / ethanol (2 : 1) at 80 °C for 1.75 h, followed by freeze-drying of the reaction mixture gave the imidazole hydrochloride salt **11**,[6] $[\alpha]_D^{25}$ -28 (c 0.1, H_2O). The sign and magnitude of the optical rotation of **11** was similar to that of THI.HCl and consistent with that predicted by Sharpless's mnemonic for the AD reaction.[15,16]

The terminal amino group of **11** could be sulfonated or acylated under basic conditions with dansyl chloride or 5-(and 6)-carboxyfluorescein succinimidyl ester (purchased from Pierce) or *N*-hydroxy-succinimido-biotin (purchased from Pierce) to give the fluorescent and biotinylated derivatives **12a-c** respectively (Scheme 3).

Scheme 3[a]



[a] Key: (a) **12a:** dansyl chloride (1 equiv.), Et_3N (4 equiv.), DMF, 0 °C, 4 h then rt. overnight, 40% yield after PTLC (10% MeOH/EtOAc). **12b:** 5-(and 6)-carboxyfluorescein succinimidyl ester (supplied by Pierce), Et_3N, DMSO, rt, overnight . **12c:** *N*-hydroxysuccinimido-biotin (supplied by Pierce), Et_3N, DMSO, rt, 4 hr (in the dark).

In summary, we have developed a new method for the synthesis of fluorescent and biotinylated derivatives of THI. We are currently using compounds **12a-c** to study the binding of THI to specific cells types. These studies will be reported at a later date.

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6. Spectral data (NMR spectra in $CDCl_3$ unless otherwise indicated) **4:** ^1H NMR δ 4.63 (t, 1H, J = 5.4 Hz, CHOH), 3.87 (brs, 1H, CHaHb), 3.80 (s, 3H, CH_3CO_2), 3.66 (brs, 1H, CHaHb), 1.62 (s, 3H, $CH_3(C)CH_3$), 1.54 (s, 3H, $CH_3(C)CH_3$), 1.48 (s, 9H, (Me_3C). MS (ES+ve) *m/z* 260.3 ($M+H^+$, 40%), 160.2 (100%). $[\alpha]_D^{23}$ +9.74 (c 0.38, $CHCl_3$). **5:** ^1H NMR δ 9.74 (d, 1H, J = 1.2 Hz, HC=O), 4.39 (ddd, 1H, J = 1.2, 6.3, 7.7 Hz, HCOCH), 3.72 (dd, 1H, J = 8.4, 17.4 Hz, CHaHb), 3.64 (dd, 1H, J = 6.6, 10.6 Hz, CHaHb), 1.58 (s, 3H, $CH_3(C)CH_3$), 1.51 (s, 3H, $CH_3(C)CH_3$), 1.45 (s, 9H, (Me_2C). **6:** ^1H NMR δ 6.35 (dd, 1H, J = 0.9, 18.9 Hz, Me_3SnCH=CH), 5.92 (dd, 1H, J = 6.3, 19.3 Hz, CH=CH), 4.45-4.35 (m, 1H, CH=CHCH), 3.66 (brs, 1H, CHaHb), 3.11 (brs, 1H, CHaHb), 1.50 (brs, 3H, $CH_3(C)CH_3$), 1.45 (brs 3H, $CH_3(C)CH_3$), 1.40 (s, 9H, (Me_3C), 0.07 (s, 9H, $(Me)_3Sn$). MS (ES+ve) *m/z* 392.2 ($M+H^+$, 100%). $[\alpha]_D^{23}$ +37.67 (c 0.29, $CHCl_3$). **8:** ^1H NMR δ 7.53 (s, 1H, H2), 6.93 (d, 1H, J = 0.9 Hz, H5), 6.59 (d, 1H, J = 15.6 Hz, imid-CH=CH), 6.31 (dd, 1H, J = 7.2, 15.6 Hz, imid-CH=CH), 5.22 (s, 2H, $EtOCH_2$), 4.62 (m, 1H, HC-O), 3.76 (brs, 1H, CHaHb), 3.42 (q, J = 7.2 Hz, $MeCH_2O$), 3.24 (t, 1H, J = 9.3 Hz, CHaHb), 1.57 (brs, 3H, CH_3), 1.53 (brs, 3H, Me), 1.46 (s, 9H, (Me_3C), 1.17 (t, 3H, J = 6.6 Hz, $MeCH_2O$). MS (ES +ve) *m/z* 352.5 ($M+H^+$, 100%). HRMS: cald. for $C_{18}H_{30}O_4N_3$, 352.22358; found 352.22389. **9:** ^1H NMR (acetone-d_6) δ 7.57 (s, 1H, H5), 6.67 (d, 1H, J = 15.6 Hz, Imid-CH=CH), 6.42 (dd, 1H, J = 6.9, 15.8 Hz, Imid-CH=CH), 5.76 (s, 2H, $EtOCH_2$), 4.75-4.67 (m, 1H, CH=CH-CH), 3.79 (dd 1H, J = 5.7, 9.75 Hz, CHaHb), 3.53 (q, 2H, J = 6.9 Hz, CH_3CH_2O), 3.18 (t, 1H, J = 9.9 Hz , CHaHb), 2.58 (s, 3H, MeCO), 1.55 (s, 3H, CH_3CCH_3), 1.52 (s, 3H, CH_3CCH_3), 1.46 (s, 9H, Me_3C), 1.12 (t, 3H, J = 7.2 Hz, CH_3CH_2O). HRMS: cald. for $C_{20}H_{22}O_5N_3$, 394.2341; found 394.2335 **10:** ^1H NMR (acetone-d_6) δ 7.49 (d, 1H, J = 0.6 Hz, H5), 5.76 (d, 2H, J = 1.2 Hz, $EtOCH_2$), 4.81 (d,1H, J = 3.3 Hz, Imid-CHOH(*1R*)), 4.27 (dd, 1H, J = 5.4, 5.1 Hz, CHOH(*2S*)), 3.91 (m, 1H, CHO), 3.68 (dd, 1H, J = 3.14, 117 Hz, CHaHb), 3.53 (q, 2H, J = 3.45 Hz, $MeCH_2O$), 3.44 (dd, 1H, J = 1.8, 10.2 Hz, CHaHb), 2.54 (s, 3H, MeCO), 1.54 (s, 3H, $CH_3(C)CH_3$), 1.46 (s, 12H, $CH_3(C)CH_3$ and (Me_3C), 1.12 (t, 3H, J = 6.9 Hz, CH_3CH_2O). HRMS: cald. for $C_{20}H_{34}O_7N_3$, 428.2396; found 428.2393. **11:** ^1H NMR (D_2O) δ 7.49 (s, 1H, H5), 5.10 (d, 1H, J = 2.1 Hz, Imid-CHOH(*1R*)), 3.91(dd, 1H, J = 3.6, 8.7 Hz, CHOH(*2S*)), 3.61 (dd, 1H, J = 2.1, 8.4 Hz, CHOH(*3R*)), 3.27 (dd, 1H, J = 3.0, 13.2 Hz, CHaHb), 2.92 (dd, 1H, J = 9.6, 13.05 Hz, CHaHb), 2.56 (s, 3H, MeCO). HRMS: cald. for $C_9H_{16}O_4N_3$, 230.11405; found 230.11491. $[\alpha]_D^{25}$ -28 (c 0.10, H_2O).

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