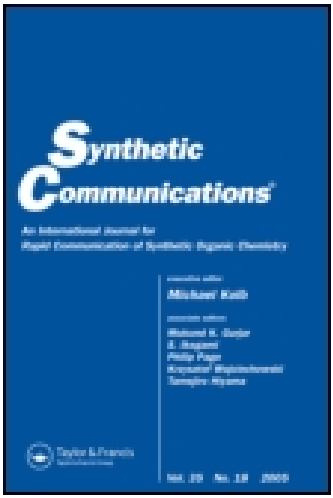

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Biotin Derivatives as Gelators of Organic Solvents

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BIOTIN DERIVATIVES AS GELATORS OF ORGANIC SOLVENTS

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ABSTRACT: The preparation of a series of biotin esters and amides is described. Several of these compounds are capable of forming stable gels with a variety of organic solvents.

INTRODUCTION

Several classes of small organic molecules that include peptides[1], anthracene derivatives[2] and calixarenes[3] have been been reported to cause gelation of common organic solvents.[4] The recovery of spilled solvents, disposal of used cooking oil and novel drug delivery systems have been suggested as possible applications for such gelling compounds. During the preparation of biotin derivatives with alkynyl side chains[5] we observed that attempted recrystalliaztion of compound **1** from ethyl acetate caused a gel to form. We then prepared a series of biotin esters and amides to probe this phenomenon and determine the scope of the gel formation in various organic solvents.

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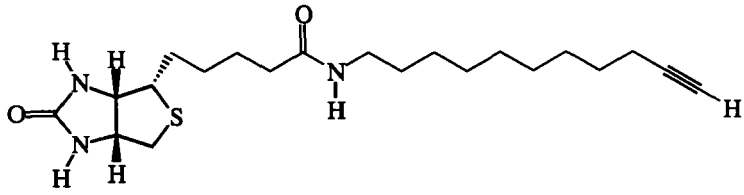

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RESULTS AND DISCUSSION

The observation that the attempted recrystallisation of biotin amide **1** from ethyl acetate gave rise to a gel was unexpected. Formation of the gel was achieved by heating **1** (about 150mg) in boiling ethyl acetate (about 35 ml) and allowing the solution to cool to room temperature. The gel was transparent, stable to mechanical inversion and retained shape upon lifting with a spatula, although vigorous treatment caused degradation of the gel to solvent and crystals of **1**. The formation of the gel was thermoreversible since reheating the solvent and crystals reformed the gel. Storage in a sealed vial prolonged the life of the gel; upon removal of the seal solvent slowly evaporated which caused the gel to shrink and a xerogel was formed. The gel did not show any structure when viewed under an optical microscope, and neither the gel nor the xerogel transmitted light when placed between crossed polarising filters (i.e. they were not birefringent). Several other solvents were tested for gelation, and the results are summarized in Table 1. Toluene formed the most mechanically stable gel with the gel being unchanged even after it was left uncovered in a fume cupboard for 30 days. Subsequent mechanical agitation of the gel resulted in the solvent being lost overnight.

^{1}H NMR spectroscopic analysis of a series of increasing concentrations of **1** in $CDCl_3$ showed the chemical shifts of the amide protons moved markedly downfield, consistent with aggregation and formation of a hydrogen bonded

Table 1. Concentration and molar gelation ratios of **1** with several solvents.

Solvent	Concentration (g/ml)	Molar ratio
Ethyl acetate	4.2	960
Benzene	3.0	1,470
Toluene	3.2	1,040
Chloroform	12.0	410

network (Table 2). The spectra were relatively well resolved, although as the concentration increased resonances were broadened slightly, and smaller poorly resolved resonances became apparent due to the hydrogen bonded network . As the solvent had gelled at these concentrations, it was anticipated that a loss of resolution would have occurred. The apparent good resolution may be explained by assuming that **1** exists in two distinct states. The first is a monomeric or intermolecularly hydrogen bonded species in solution which rotates rapidly and so exhibits well resolved resonances, and the second is a hydrogen bonded network whose resonances are broadened due to the restricted rotation. The spectra at higher concentrations are superpositions of the two states.

Table 2. Chemical shift of amide protons of **1** vs concentration.

Concentration[a]	N1'-H	N3'-H	CH_2NHCO
0.5mg	4.56	5.12	5.45
2.0mg	4.85	5.57	5.59
5.0mg[b]	5.13	5.95	5.73
10.0mg[b]	5.31	6.16	5.82
25.0mg[b]	5.35	6.19	5.84

([a]per 0.6ml $CDCl_3$; [b]gel formed)

The relatively well resolved spectra in $CDCl_3$ were in contrast to the spectrum of a [$^2H_8$]-toluene gel which was poorly resolved at room temperature. Increasing the temperature of the sample in 20° steps showed the resolution improved between 80° and 100°, presumably when the gelation temperature (T_{gel}) was exceeded.

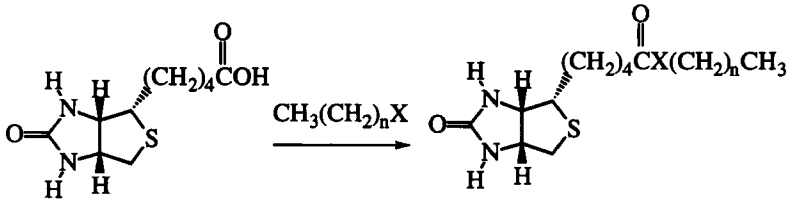
Increasing the temperature of a 10mg/0.6ml gel in $CDCl_3$ showed large upfield changes in the chemical shifts (Table 3) and broadening of the resonances for the amide protons, consistent with disruption in the hydrogen bonding which exists between the presumed dimeric, trimeric etc. associations of molecules.

Table 3. Chemical shift of amide protons of **1** (δ ppm) vs Temperature (K)

T (K)	N1'-H	N3'-H	CH_2NHCO
293	5.62	6.23	5.91
303	5.23	6.01	5.82
313	5.09	5.79	5.71
323	4.97	5.61	5.61

The possible uses of compounds which gelate organic solvents may necessitate the inclusion of low polarity solvents such as aliphatic hydrocarbons. The amide **1** was not soluble in hexane,and so a series of biotin amides and biotin esters with varying alkyl chain lengths (*n*-propyl, *n*-hexyl, *n*-octyl, *n*-undecyl, *n*-dodecyl, *n*-hexadecyl) were prepared in order to determine the scope of gelation of solvents by biotin derivatives. Reaction of biotin NHS ester with an amine in DMF (Scheme, Condition A) gave the amides, **2-7**, in good yields. The reactions were generally complete within 2 hours and workup consisted of removal of the precipitated NHS by filtration, removal of the solvent *in vacuo*, chromatography and recrystallisation to give the amides as colorless, amorphous solids. Biotin esters, **8-13**, were prepared by reaction of biotin with 5 equivalents of alcohol in toluene heated to reflux, catalysed by *p*-toluenesulphonic acid (Scheme, Condition B). Heating at reflux for 48 hours, removal of the solvent *in vacuo*, filtration to remove unreacted biotin, and chromatography gave the esters in poor to excellent yields. The undec-10-yn-1-ol ester **14** (the ester analogue of **1**)was prepared by the reaction of biotin NHS ester with undec-10-yn-1-ol in DMF, catalysed by DMAP.

Gelation tests were performed by placing a weighed amount of compound (15-20mg) in a preweighed vial and heating with the solvent to be tested. After dissolution (if not insoluble) and cooling to room temperature for 60 minutes the outcome of the experiment was noted.



2 - 7 X = NH
8 - 13 X = O

Condition A: (i) NHS, DCC, DMF, 80° to RT, 2 hours. (ii) $CH_3(CH_2)_nNH_2$.
2 (n = 15); **3** (n = 11); **4** (n = 10); **5** (n = 7); **6** (n = 5); **7** (n = 2)

Condition B: $CH_3(CH_2)_nOH$, *p*-TsOH, toluene, reflux 48 hours.
8 (n = 15); **9** (n = 11); **10** (n = 10); **11** (n = 7); **12** (n = 5); **13** (n = 2)

Scheme

A gel was considered to have formed if a transparent mass which did not flow upon inversion of the vial was observed. The total mass of the vial and solvent was determined and the concentration of gelator calculated. Gelation was generally complete by the time room temperature was reached; more vigorous attempts to achieve gelation such as cooling the gel in cold water or a refrigerator were not attempted. If the compound was soluble at room temperature, the amount of solvent was reduced by evaporation and the concentrated solution studied for gelation as above. The results of the gelation tests are shown in Table 4 for the amides and Table 5 for the esters.

The results of the gelation tests showed (i) the optimal length of the alkyl substituent is between 8 and 12 carbons (*n*-octyl to *n*-dodecyl), i.e. an overall (including pentanoic chain of biotin and heteroatom) length of 14 to 18 atoms; (ii) the *n*-

the *n*-dodecyl, *n*-undecyl and *n*-octyl esters (**9**, **10** and **11** respectively) gel hexane when predissolved in CH_2Cl_2; (iii) the octyl ester **10** gelled light paraffin oil when predissolved in CH_2Cl_2, however crystallisation occurred after 14 days; (iv) a terminal alkyne is important in enhancing the gelation process.

Table 4. Gelation of Solvents by Biotin Amide Derivatives

	2	**3**	**4**	**5**	**6**	**7**	**1**
CCl_4	s	c	c	c	i	i	i
$CHCl_3$	s	18	21	22	c	c	12
CH_2Cl_2	i	c	c	c	i	i	i
EtOAc	i	c	c	c	c	c	4.2
hexanes	i	i	i	i	i	i	i
toluene	s	c	c	c	c	i	3.2
acetonitrile	c	c	c	c	c	c	c
ether	s	i	i	i	i	i	i
MeOH	c	c	c	s	s	c	c
acetone	i	c	c	c	c	c	c
benzene	nm	nm	nm	nm	nm	nm	3

i insoluble, *c* crystallise, *s* soluble, *nm* not measured, number = conc (g/ml) of gel

Table 5. Gelation of Organic Solvents by Biotin Esters

	8	**9**	**10**	**11**	**12**	**13**	**14**
CCl_4	c	s	s	43	9	17	s
$CHCl_3$	s	s	s	s	s	s	s
CH_2Cl_2	s	s	s	s	s	s	s
EtOAc	c	s	s	s	s	s	s
hexanes	i	c	c	i	i	i	i
toluene	c	s	s	s	s	s	s
acetonitrile	c	c	s	s	s	s	s
ether	i	c	i	i	i	i	i
MeOH	s	s	s	s	s	s	s
acetone	c	s	s	s	s	s	s
CH_2Cl_2/ hexane	c	5.3	c	9.9	c	c	*
paraffin	nm	nm	nm	2.4	nm	nm	nm

*formed a transparent polymeric mass
i insoluble, *c* crystallise, *s* soluble, *nm* not measured, number = conc (g/ml) of gel

Terminal alkynes are known to undergo weak hydrogen bonding[6], however the chemical shift of the acetylenic proton showed no significant change in either the variable concentration or variable temperature experiments. How the alkyne enhances the gelation process is unclear at this stage. As the undecylamide **3** is not as efficient a gelator as **1** small differences in structure can lead to large differences in gelator behaviour. Further work is required to determine the structure of the hydrogen bonded network on both the molecular and macroscopic scales for this new class of thermoreversible gelator compounds. In conclusion, the compounds are readily prepared, gel at low concentrations, may form very stable gels and can be designed to gel hexane.

EXPERIMENTAL

Melting points were recorded on a Reichhert hot stage apparatus and are uncorrected. Proton and carbon NMR spectra were recorded on a Bruker ACP-300 or a Varian Gemini 200 spectrometer. $CDCl_3$ was used as a solvent unless otherwise stated, with tetramethylsilane used as an internal standard. Mass spectra were recorded on VG ZAB 2HF mass spectrometer with either electron impact (EI) or fast atom bombardment (FAB) ionisation, or on an AEI-GEC MS 3074 instrument with EI ionisation. Accurate mass determinations using EI or Liquid Secondary Ion MS (LSIMS) were made by the Organic Mass Spectrometry Facility at the University of Tasmania, or using EI at the Department of Chemistry, University of Melbourne. CH_2Cl_2 and DMF were distilled from CaH_2 under nitrogen and stored over 4Å molecular sieves. Other regents were purified according to standard literature procedures.[7] Analytical thin layer chromatography was carried out using Merck aluminium sheets precoated with kieselgel 60 F_{254}, or with a 4% solution of phosphomolybdic acid in ethanol. Flash chromatography was carried using Merck kieselgel 60 (230-400 mesh) and solvents used were distilled before use.

Biotin-*N*-(1-hexadecyl)amide (2)

To a stirred solution of biotin-NHS ester[8] (100mg, 0.29 mmol) in DMF (2ml) was added 1-hexadecylamine (71mg, 0.29 mmol). A white precipitate formed within 10

minutes. The mixture was stirred overnight, the solvent removed *in vacuo* (oil pump), the residue purified by flash chromatography using 10/90 MeOH/CH_2Cl_2 as eluant, recrystallised from MeOH and dried under vacuum to give the title compound as a colorless solid in 115mg (83%) mp 196-198°. HRMS: Calculated for $C_{26}H_{49}N_3O_2S$: 467.3544. Found: 467.3536. MS: 467 (M^+, 34), 450 (22), 407 (44), 166 (29), 97 (37), 43 (100). ^1H NMR: 0.88 (*t*, 3H, *J* 6.5 Hz, CH_3-CH_2); 1.25-1.77 (*m*, methylene protons); 2.20 (*t*, 2H, J 6.2 Hz, CH_2-CON); 2.74 (*d*, 1H, J_{gem} 12.8 Hz, C5-Hβ); 2.93 (*dd*, 1H, J_{gem} 12.8, 5.0 Hz, C5-Hα); 3.17 (*dt*, 1H, *J* 7.3, 4.7 Hz, C2-H); 3.23 (*q*, 2H, *J* 6.6 Hz, CH_2-NHCO); 4.33 (*dd*, 1H, *J* 7.7, 5.0 Hz, C4-H); 4.52 (*dd*, 1H, *J* 7.7 Hz, 4.7 Hz, C3-H); 4.91 (bs, 1H, N1'-H); 5.63 (*bs*, 1H, N3'-H); 5.65 (*bt*, 1H, *J* 5.3 Hz, -NHCO). ^{13}C (CD_3OD, 323K): 14.2, 23.6, 26.8, 28.0, 29.5, 29.7, 30.3, 30.4, 30.6, 30.6, 32.9, 36.9, 40.5, 41.0, 56.9, 61.7, 63.5, 175.9, 184.9.

Compounds **3**, **4**, **5**, **6** and **7** were prepared in a similar manner, varying only in the amine used, product yield and recrystallisation solvent.

Biotin-*N*-(1-dodecyl)amide (3)

Recrystallised from MeOH. Recovered 86mg (71%) of colorless amorphous solid mp 193-196° (lit.[9]194-198°). HRMS Calculated for $C_{22}H_{41}N_3O_2S$: 411.2919. Found: 411.2904. MS: 411 (M^+, 13), 351 (100), 227 (68), 186 (61), 166 (77), 148 (90). ^1H ($CDCl_3$/d_6-DMSO): 0.89 (*t*, 3H, J 6.4 Hz, CH_3-CH_2); 0.96-1.47 (*m*, methylene protons); 1.89, *t*, 2H, *J* 7.4 Hz, CH_2CONH); 2.44 (*d*, 1H, J_{gem} 12.7 Hz, C5-Hb); 2.60 (*dd*, 1H, J_{gem} 12.7, 4.9 Hz, C5-Hα); 2.82-2.90 (*m*, 3H, CH_2CON and C2-H); 3.97 (*dd*, 1H, *J* 7.8, 4.9 Hz, C4-H); 4.45 (*dd*, 1H, *J* 7.8 Hz, 4.8 Hz, C3-H); 5.53 (*bs*, 1H, N1'-H); 5.56 (*bs*, 1H, N3'-H); 6.51 (*bt*, 1H, *J* 5.2 Hz, -NHCO). ^{13}C (d_6-DMSO): 14.0, 22.1, 25.4, 26.5, 28.1, 28.2, 28.7, 28.8, 29.1, 29.2, 31.3, 35.3, 38.6, 40.7, 55.8, 59.2, 61.1, 162.7, 171.8 .

Biotin-*N*-(1-undecyl)amide (4)

Recrystallised from MeOH. Recovered 65mg (56%) of colorless amorphous solid mp 183-186°. HRMS Calculated for $C_{21}H_{39}N_3O_2S$: 397.2763. Found: 397.2758. MS: 397 (M^+, 10), 337 (100), 226 (49), 172 (55), 166 (75), 97 (72). ^1H NMR: 0.89 (*t*, 3H, *J* 6.4 Hz, CH_3-CH_2-); 1.26-1.77 (*m*, methylene protons); 2.21 (*t*, 2H, *J* 7.5 Hz, CH_2-CON); 2.74 (*d*, 1H, J_{gem} 12.8 Hz, C5-Hβ); 2.93 (*dd*, 1H, J_{gem} 12.8, 4.9 Hz, C5-Hα); 3.16 (*dt*, 1H, *J* 7.3, 4.8 Hz, C2-H); 3.16 (*dt*,

1H, *J* 7.3, 4.8 Hz, C2-H); 3.23 (*q*, 2H, *J* 6.3 Hz, CH_2-NHCO); 4.33 (*dd*, 1H, *J* 7.6, 4.6 Hz, C4-H); 4.52 (*dd*, 1H, *J* 7.6 Hz, 4.9 Hz, C3-H); 5.16 (*bs*, 1H, N1'-H); 5.77 (*bt*, 1H, *J* 5.4 Hz, -NHCO); 5.98 (*bs*, 1H, N3'-H). ^{13}C NMR: 14.1, 22.7, 25.4, 25.6, 27.0, 28.0, 28.1, 29.3, 29.6, 29.1, 31.9, 36.0, 39.6, 40.5, 55.4, 60.2, 61.8, 163.7, 173.0.

Biotin-*N*-(1-octyl)amide (5)

Recrystallised from MeOH/water. Recovered 70mg (67%) of colorless amorphous solid mp 193-196°. HRMS Calculated for $C_{18}H_{33}N_3O_2S$: 355.2293. Found: 355.2293. MS: 355 (M^+, 7), 338 (5), 311 (9), 295 (100), 227 (30), 184 (56), 166 (55), 130 (68), 100(64). ^{1}H NMR: 0.88 (*t*, 3H, *J* 6.6 Hz, CH_3-CH_2); 1.28-1.77 (*m*, 18H, methylene protons); 2.20, *t*, 2H, *J* 7.3 Hz, CH_2-CON); 2.74 (*d*, 1H, J_{gem} 12.8 Hz, C5-Hβ); 2.93 (*dd*, 1H, J_{gem} 12.8, 4.9 Hz, C5-Hα); 3.17 (*dt*, 1H, *J* 7.2, 4.7 Hz, C2-H); 3.23 (*q*, 2H, *J* 7.1 Hz, CH_2-NHCO); 4.34 (*dd*, 1H, *J* 7.7, 4.9 Hz, C4-H); 4.53 (*dd*, 1H, *J* 7.7 Hz, 4.7 Hz, C3-H); 4.92 (*bs*, 1H, N1'-H); 5.63 (*bs*, 2H, N3'-H and -NHCO). ^{13}C NMR: 14.1, 22.6, 25.6, 26.9, 28.1, 29.3, 29.5, 29.6, 31.7, 31.8, 36.0, 39.6, 40.5, 55.4, 60.1, 61.8, 163.4, 173.0.

Biotin-*N*-(1-hexyl)amide (6)

Recrystallised from MeOH. Recovered 63mg (66%) of colorless morphous solid mp 183-186°. HRMS Calculated for $C_{16}H_{29}N_3O_2S$: 327.1980. Found: 327.1971. MS: 327 (M^+, 2), 310 (3), 283 (4), 267 (40), 184 (68), 166 (23), 156 (28), 143 (26), 116 (22), 100 (100). ^{1}H NMR: 0.89 (*t*, 3H, J 6.7 Hz, CH_3-CH_2); 1.29-1.80 (*m*, 14H, methylene protons); 2.20 (*t*, 2H, *J* 7.4 Hz, CH_2-CON); 2.74 (*d*, 1H, J_{gem} 12.9 Hz, C5-Hβ); 2.93 (*dd*, 1H, J_{gem} 12.9, 5.0 Hz, C5-Hα); 3.17 (*dt*, 1H, *J* 7.1, 4.6 Hz, C2-H); 3.23 (*q*, 2H, *J* 7.0 Hz, CH_2-NHCO); 4.34 (*dd*, 1H, *J* 7.6, 5.0 Hz, C4-H); 4.45 (*dd*, 1H, *J* 7.6 Hz, 4.6 Hz, C3-H); 4.85 (*bs*, 1H, N1'-H); 5.54 (*bs*, 1H, N3'-H); 5.63 (*bt*, 1H, *J* 5.2 Hz, -NHCO). ^{13}C NMR (d_6-DMSO): 13.8, 22.0, 25.2, 26.0, 27.9, 28.1, 29.0, 30.9, 35.1, 38.2, 38.4, 55.3, 59.1, 60.9, 162.6, 171.6.

Biotin-*N*-(1-propyl)amide (7)

Recrystallised from water to give a colorless amorphous solid mp 193-194° (lit.[10] 195-200°) in 61mg (73%) yield. HRMS Calculated for $C_{13}H_{23}N_3O_2S$: 285.1511. Found: 285.1511. MS: 285 (M^+, 4), 225 (67), 166 (26), 142 (45), 114 (39), 100 (100). ^{1}H NMR: 0.92 (*t*, 3H, CH_3-CH_2-); 1.41-1.83 (*m*, 8H, methylene protons);

2.21 (*t*, 2H, *J* 7.4 Hz, CH_2-CONH); 2.75 (*d*, 1H, J_{gem} 12.9 Hz, C5-Hβ); 2.83 (*dd*, 1H, J_{gem} 12.9, 5.0 Hz, C5-Hα); 3.16 (*dt*, 1H, *J* 7.4, 4.8 Hz, C2-H); 3.21 (*q*, 2H, *J* 7.0 Hz, CH_2-NHCO); 4.34 (*dd*, 1H, *J* 7.6, 5.0 Hz, C4-H); 4.45 (*dd*, 1H, *J* 7.6 Hz, 4.8 Hz, C3-H); 5.12 (*bs*, 1H, N1'-H); 5.72 (*bt*, 1H, *J* 5.2 Hz, -NHCO); 5.83 (*bs*, 1H, N3'-H). ^{13}C NMR (d_6-DMSO): 11.7, 23.6, 27.0, 29.5, 29.8, 36.9, 41.0, 42.2, 47.8, 57.0, 61.7, 63.4, 166.1, 176.0.

Biotin hexadecan-1-ol ester (8)

A mixture of biotin (100mg, 0.41 mmol), hexadecan-1-ol (496mg, 2.05 mmol), *p*-toluenesulphonic acid (8mg, 0.04 mmol) and toluene (5ml) was stirred at reflux for 48 hours. The reaction mixture was cooled to room temperature, unreacted biotin removed by filtration, solvent removed *in vacuo* and the residue purified by flash chromatography, eluant MeOH/CH_2Cl_2 5:95, to give the title compound as a colorless solid in 72mg (38%) yield mp 113-118°. HRMS Calculated for $C_{26}H_{48}N_2O_3S$: 468.3386. Found: 468.3369. MS: 468 (M^+, 100), 296 (20), 227 (22), 97 (24). ^1H NMR: 0.90 (*t*, 3H, *J* 6.4 Hz, CH_3-CH_2); 1.28-1.77 (*m*, methylene protons); 2.35 (*t*, 2H, *J* 7.2 Hz, CH_2-CO_2); 2.76 (*d*, 1H, J_{gem} 12.8 Hz, C5-Hβ); 2.95 (*dd*, 1H, J_{gem} 12.8, 5.0 Hz, C5-Hα); 3.19 (*dt*, 1H, *J* 8.0, 4.7 Hz, C2-H); 4.08 (*t*, 2H, *J* 6.8 Hz, CH_2-O_2C); 4.34 (*dd*, 1H, *J* 7.5, 4.5 Hz, C4-H); 4.54 (*dd*, 1H, *J* 7.5, 5.0 Hz, C3-H); 4.88 (*bs*, 1H, N1'-H); 5.18 (*bs*, 1H, N3'-H). ^{13}C NMR: 14.1, 22.7, 24.8, 25.9, 28.2, 28.3, 28.6, 29.3, 29.4, 29.5, 29.6, 29.6, 29.7, 31.9, 33.9, 40.5, 55.4, 60.1, 61.9, 64.6, 163.4, 173.8.

Biotin esters **9**, **10**, **11** and **12** were prepared in a similar manner, varying only in the alcohol used and yield obtained.

Biotin dodecan-1-ol ester (9)

Recovered in 136mg (80%) yield as a colorless amorphous solid mp 117-118°. HRMS Calculated for $C_{22}H_{40}N_2O_3S$: 412.2760. Found: 412.2761. MS: 412 (M^+, 4), 352 (15), 227 (40), 166 (100), 143 (16). ^1H NMR: 0.89 (*t*, 3H, *J* 6.6 Hz, CH_3-); 1.27-1.76 (*m*, methylene protons); 2.34 (*t*, 2H, *J* 7.6 Hz, CH_2-CONH); 2.75 (*d*, 1H, *J* 12.8 Hz, C5-Hβ); 2.93 (*dd*, 1H, *J* 12.8, 4.9 Hz, C5-Hα); 3.17 (*dt*, 1H, *J* 7.9, 4.8 Hz, C2-H); 4.06 (*t*, 2H, *J* 6.8 Hz, CH_2-O_2C); 4.32 (*dd*, 1H, *J* 7.4, 5.0 Hz, C3-H); 4.52 (*dd*, 1H, *J* 7.4, 5.0 Hz, C4-H); 5.15 (*bs*, 1H, N1'-H); 5.51 (*bs*, 1H, N3'-H). ^{13}C NMR: 14.1, 22.7, 24.8, 25.9, 28.2, 28.3, 28.6, 29.2, 29.3, 29.5, 29.6, 29.6, 31.9, 33.9, 40.6, 55.4, 60.2, 61.9, 64.6, 165.0, 173.8.

Biotin-undecan-1-ol ester (10)

Recovered in 129mg (79%) yield as a colorless amorphous solid mp 110-113°. HRMS: Calculated for $C_{21}H_{38}N_2O_3S$: 398.2603. Found: 398.2584. MS: 398 (M^+, 5), 338 (24), 227 (24), 166 (100), 97 (46). ^1H NMR: 0.91 (3H, *J* 6.4 Hz, CH_3-CH_2); 1.29-1.77 (*m*, methylene protons); 2.36 (*t*, 2H, *J* 7.3 Hz, CH_2-CO_2); 2.76 (*d*, 1H, J_{gem} 12.8 Hz, C5-Hβ); 2.96 (*dd*, 1H, J_{gem} 12.8, 4.9 Hz, C5-Hα); 3.19 (*dt*, 1H, *J* 7.6, 4.9 Hz, C2-H); 4.09 (*t*, 2H, *J* 6.8 Hz, CH_2-O_2C); 4.35 (*dd*, 1H, *J* 6.6, 4.6 Hz, C4-H); 4.52 (*dd*, 1H, *J* 6.6, 4.9 Hz, C3-H); 4.66 (*bs*, 1H, N1'-H); 4.88 (*bs*, 1H, N3'-H). ^{13}C NMR: 14.1, 22.6, 24.8, 25.9, 28.2, 28.3, 28.6, 29.2, 29.3, 29.5, 29.5, 31.8, 33.9, 40.5, 55.4, 60.1, 61.9, 64.5, 163.7, 173.8.

Biotin octan-1-ol ester (11)

Recovered 147mg (98%) as a colorless amorphous solid mp 121°. HRMS Calculated for $C_{18}H_{32}N_2O_3S$: 356.2134. Found: 356.2128. MS: 356 (M^+, 100), 329 (26), 287 (51), 227 (52), 166 (82), 97 (71). ^1H NMR: 0.89 (*t*, 3H, *J* 6.5 Hz, CH_3-CH_2); 1.28-1.76 (*m*, 18H, methylene protons); 2.35 (*t*, 2H, *J* 7.2 Hz, CH_2-CO_2); 2.74 (*d*, 1H, J_{gem} 12.8 Hz, C5-Hβ); 2.92 (*dd*, 1H, J_{gem} 12.8, 4.7 Hz, C5-Hα); 3.16 (*dt*, 1H, *J* 6.9, 4.8 Hz, C2-H); 4.06 (*t*, 2H, *J* 6.8 Hz, CH_2-O_2C); 4.32 (*dd*, 1H, *J* 7.7, 4.8 Hz, C4-H); 4.52 (*dd*, 1H, *J* 7.7, 4.8 Hz, C3-H); 5.21 (*bs*, 1H, N1'-H); 5.59 (*bs*, 1H, N3'-H). ^{13}C NMR: 14.1, 22.7, 24.8, 25.9, 28.2, 28.3, 28.6, 29.3, 29.4, 29.5, 29.6, 29.6, 29.7, 31.9, 33.9, 40.5, 55.4, 60.1, 61.9, 64.6, 163.4, 173.8.

Biotin hexan-1-ol ester (12)

Recovered 110mg (81%) as a colorless amorphous solid mp 119-120°. HRMS Calculated for $C_{16}H_{28}N_2O_3S$: 328.1821. Found: 328.1827. MS: 328 (M^+, 19), 268 (14), 227 (19), 166 (39), 105 (100). ^1H NMR: 0.89 (*t*, 3H, *J* 6.3 Hz, CH_3-CH_2); 1.29-1.71 (*m*, 14H, methylene protons); 2.33 (*t*, 2H, *J* 7.0 Hz, CH_2-CO_2); 2.73 (*d*, 1H, J_{gem} 12.8 Hz, C5-Hβ); 2.91 (*dd*, 1H, J_{gem} 12.8, 4.8 Hz, C5-Hα); 3.15 (*dt*, 1H, *J* 7.4, 4.8 Hz, C2-H); 4.05 (*t*, 2H, *J* 6.7 Hz, CH_2-O_2C); 4.30 (*dd*, 1H, *J* 7.7, 4.8 Hz, C4-H); 4.51 (*dd*, 1H, *J* 7.7, 4.8 Hz, C3-H); 5.38 (*bs*, 1H, N1'-H); 5.78 (*bs*, 1H, N3'-H). ^{13}C NMR: 13.9, 22.5, 24.8, 25.5, 28.2, 28.4, 28.6, 31.4, 33.9, 40.5, 55.4, 60.1, 61.9, 64.5, 163.8, 173.8.

Biotin propan-1-ol ester (13)

A mixture of biotin (100mg, 4.1 mmol), propan-1-ol (3ml) and *p*-toluene sulfonic acid (7.8mg, 0.04 mmol) was refluxed for 6 hours, at which time a homogeneous mixture was formed. The reaction mixture was cooled to room temperature, crystallised unreacted biotin removed by filtration, the solvent removed *in vacuo* and the residue purified by flash chromatography eluant MeOH/CH_2Cl_2 5:95 to give the title compound as colorless crystals mp 126-127° in 73mg (62 %) yield. HRMS: Calculated for $C_{13}H_{22}N_2O_3S$: 286.1351. Found: 286.1362. MS: 286 (M^+, 7), 227 (82), 166 (84), 97 (100). ^1H NMR: 0.95 (*t*, 3H, *J* 7.5 Hz, CH_3-CH_2); 1.43-1.78 (*m*, 8H, methylene protons); 2.34 (*t*, 2H, *J* 7.3 Hz, CH_2-CO_2-); 2.74 (*d*, 1H, J_{gem} 12.8 Hz, C5-Hβ); 2.93 (*dd*, 1H, J_{gem} 12.8, 4.9 Hz, C5-Hα); 3.17 (*dt*, 1H, *J* 8.0, 4.7 Hz, C2-H); 4.04 (*t*, 2H, *J* 6.8 Hz, CH_2-O_2C); 4.33 (*dd*, 1H, *J* 7.6, 4.7 Hz, C4-H); 4.53 (*dd*, 1H, *J* 7.6, 4.7 Hz, C3-H); 5.00 (*bs*, 1H, N1'-H); 5.34 (*bs*, 1H, N3'-H). ^{13}C NMR: 10.3, 21.9, 24.7, 28.2, 28.3, 33.9, 40.5, 55.4, 60.1, 61.9, 65.9, 163.9, 173.8.

Biotin-undec-10-yn-1-ol ester (14)

A mixture of biotin-NHS ester (100mg, 0.29 mmol), undec-10-yn-1-ol[11] (49mg, 0.29 mmol) and DMAP (3.6mg, 0.03 mmol) was stirred in DMF (0.5ml) for 48 hours at 50°. Silica gel (1g) was added and the solvent removed under vacuum (oil pump). The residue was purified by flash chromatography eluant MeOH/CH_2Cl_2 6/94 to give the title compound as a colorless amorphous solid mp 84-86° in 52.6mg (46%) yield. HRMS Calculated for $C_{21}H_{34}N_2O_3S$: 394.2290. Found: 394.2298. MS: 394 (M^+, 6), 334 (18), 227 (62), 166 (37), 97 (100). ^1H NMR: 1.25-1.77 (*m*, methylene protons); 1.94 (*t*, 1H, *J* 2.6 Hz, H-C≡C); 2.18 (*dt*, 1H, *J* 6.8, 2.6 Hz, CH_2-C≡C); 2.29 (*t*, 2H, *J* 7.1 Hz, CH_2-CO_2); 2.74 (*d*, 1H, *J* 12.8 Hz, C5-Hβ); 2.92 (*dd*, 1H, *J* 12.8, 4.8 Hz, C5-Hα); 3.16 (*dt*, 1H, *J* 7.4, 4.7 Hz, C2-H); 4.05 (*t*, 2H, *J* 6.6 Hz, CH_2-O_2C); 4.31 (*dd*, 1H, *J* 7.6, 4.7 Hz, C4-H); 4.51 (*dd*, 1H, *J* 7.6, 4.8 Hz, C3-H); 5.43 (*bs*, 1H, N1'-H); 5.81 (*bs*, 1H, N3'-H). ^{13}C NMR: 20.4, 26.8, 27.9, 30.3, 30.4, 30.5, 30.6, 30.7, 31.0, 31.2, 31.3, 35.9, 42.5, 57.4, 62.1, 64.0, 66.6, 70.1, 86.7, 165.5, 175.7.

REFERENCES

1. (a) Hanabusa, K., Tange, J., Taguchi, Y. and Koyama, T., Shirai, H. *J. Chem. Soc., Chem. Commun.* **1993**, 390. (b) Hanabusa, K., Naka, Y.,

Koyama, T. and Shirai, H. *J. Chem. Soc., Chem. Commun.* **1994**, 2683. (c) Hanabusa, K., Miki, T., Taguchi, Y., Koyama, T. and Shirai, H. *J. Chem. Soc., Chem. Commun.* **1993**, 1382. (d) De Vries, E. J. and Kellog, R. M. *J. Chem. Soc., Chem. Commun.* **1993**, 238. (e) Hanabusa, K., Okui, K., Karaki, K., Koyama, T., and Shirai, H. *J. Chem. Soc., Chem. Commun.* **1992**, 1371.

2. Brotin, T., Utermohlen, R., Fages, F., Bouas-Laurent, H. and Desvergne, J.-P. *J. Chem. Soc., Chem. Commun.* **1991**, 416.
3. (a) Aoki, M., Nakashima, K., Kawabata, H., Tsutsui, S. and Shinkai, S. *J. Chem. Soc. Perkin Trans. 2* **1993**, 347; (b) Aoki, M., Murata, K. and Shinai, S. *Chem. Lett.* **1991**, 1715.
4. Stock, H. T., Turner, N. J. and McCague, R. *J. Chem. Soc., Chem. Commun.* **1995**, 2063.
5. Crisp, G.T. and Gore, J. *Tetrahedron* in press.
6. Pilkington, M., Wallis,J.D., Smith,G.T. and Howard, J.A.K. *J.Chem. Soc., Perkin Trans. 2* **1996**, 1849.
7. Perrin, D.D., Armarego, W.L.F. and Perin, D.R. Purification of Laboratory Chemicals, 2nd Ed **1980** Pergammon Press.
8. Coughlin, R. W. and Baclaski, J. B. *Biotechnol. Prog.* **1990**, *6*, 307.
9. McCormick, D. B. *Heterocycles* **1973**, 235.
10. (a) Oserko, A. *Chem. Ber.* **1937**, *70B*, 55, (CA **1937**, 31, 2580). (b) Oehlschlager, A. C.; Czyzewska, E.; Aksela, R.; Pierce Jr. and H. D. *Can. J. Chem.* **1986**, *64*, 1407-1413.

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