A Facile One-Pot Synthesis of 3,4-Dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine and Derivatives

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A new one-pot synthesis of 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (Dhbt-OH) and derivatives directly from anthranilic acids is described. The reaction of anthranilic acids and thionyl chloride affords unstable sulfinamide anhydrides which are treated with *O*-(trimethylsilyl)hydroxylamine followed by diazotization to give the corresponding Dhbt-OH derivatives in good overall yield.

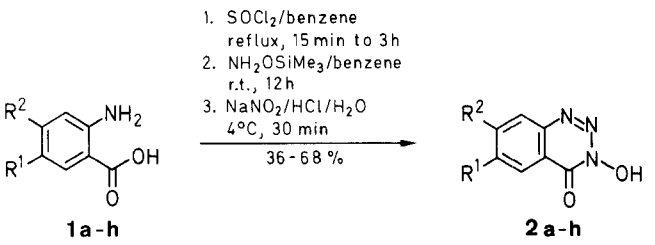
3,4-Dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (Dhbt-OH, **2a**) is an advantageous additive in the synthesis of peptides using either the carbodiimide or the active ester method. It suppresses racemization and the resulting peptides are generated in high yields and with high purity. This was first recognized by König and Geiger in 1970[1,2] who also published the first preparation of Dhbt esters of α-amino protected amino acids. However, the formation of a 2-azidobenzoic acid Dhbt ester as a byproduct during preparation of the activated amino acid ester has limited the use of these compounds in peptide synthesis. The azidobenzoate itself is an efficient acylating agent, and its presence in the acylating mixture leads to the formation of deletion peptides. This limitation was overcome in 1986 when the synthesis of pure crystalline 9-fluorenylmethoxycarbonyl (Fmoc) amino acid derivatives of Dhbt-OH was published.[3]

Most of the Fmoc amino acid derivatives of Dhbt-OH are stable crystalline substances. Their reaction rates with nitrogen nucleophiles in dimethylformamide are similar to those of symmetrical anhydrides.[3,4] In addition to their high reactivity they act as self indicating esters owing to the ionization of Dhbt-OH released into solution by unreacted resin bound amino groups.[3] This produces a transient yellow color on the resin which fades upon completion of the acylation reaction, thus acting as a sensitive indicator of resin bound free amino groups. By monitoring the transmission of light through the resin at 440 nm and feeding the data into a computer the individual coupling times could be determined and automatic termination of the acylation steps was effectuated.[5,6]

We have constructed an automated peptide synthesizer, including a solid-phase photometer, which utilizes such a monitoring system, and wished to investigate the substituent effect of Dhbt-OH as an acid–base indicator and on acylation rates.

Besides the parent compound, only the 6-iodo derivative of Dhbt-OH had previously been prepared and tested in peptide synthesis.[1] This was done by introducing a hydroxamic acid function via the methyl ester of anthranilic acid or 5-iodoanthranilic acid, followed by diazotization.[7,8] The method is cumbersome since it is necessary to isolate the methyl ester. We have explored alternative synthetic routes to Dhbt-OH and its derivatives using anthranilic acids as starting materials. Activation of the carboxylic acid function could be achieved by either phosgene or thionyl chloride with simultaneous protection of the amino group as in the reaction of anthranilic acids and phosgene to isatoic anhydrides[9] whose reactions with amines and various nucleophiles have been examined.[9,10,11] Isatoic anhydride affords only one product with hydroxylamine, which was originally believed to be 2-aminobenzhydroxamic acid,[16] but later shown to be *O*-(2-aminobenzoyl)hydroxylamine.[7]

Thionyl chloride reacts with anthranilic acid (**1a**) in an analogous manner giving an unstable sulfinamide anhydride.[12] The sulfinamide anhydride is more reactive than isatoic anhydride and treatment with *O*-(trimethylsilyl)hydroxylamine led to formation of the trimethylsilylated hydroxamic acid which was hydrolyzed, diazotized and ring closed to give the corresponding Dhbt-OH (**2a**) (Scheme). We have now developed this reaction into a new facile one-pot synthesis giving the Dhbt-OH and derivatives in good yield.



1, 2	R^1	R^2	1, 2	R^1	R^2
a	H	H	**e**	H	NO_2
b	I	H	**f**	Me	Me
c	Me	H	**g**	OMe	OMe
d	H	Cl	**h**	$-CH=CH-CH=CH-$	

Scheme

In a typical procedure the anthranilic acid derivative **1** is suspended in dry benzene or a mixture of dry benzene and dry tetrahydrofuran, thionyl chloride is added, and the mixture refluxed until a clear solution is obtained (15 min–3 h). The solvent and residual thionyl chloride are evaporated, the sulfinamide anhydride is suspended in benzene, and *O*-(trimethylsilyl)hydroxylamine is added. After evaporation of the solvent the resultant hydroxamic acids are diazotized in dilute hydrochloric acid to give the crude Dhbt-OH or its derivatives. After workup Dhbt-OH or its derivatives are isolated in 36%–68% yield calculated from the starting anthranilic acids.

Two of the new compounds, **2e** and **2h**, showed interesting spectroscopic properties for the monitoring of acylation reactions during solid-phase peptide synthesis. Both gave deep red solutions in dimethylformamide and **2e** has been tested as an additive in the synthesis of peptides. Used as an additive during solid phase peptide synthesis

using Fmoc-amino-acid-OPfp-esters compound **2e** gave a deep red color to the resin which faded to faint yellow on completion of the coupling reactions. This allows an enhanced visual detection of reaction end points compared to that of Dhbt-OH itself. Compound **2h** shows a strong pH dependent fluorescence in ethanol and its possible use as a fluorescence indicator during solid-phase peptide synthesis will be examined.

All anthranilic acids were of commercial quality and, except for 3-amino-2-naphthoic acid (**1h**), dried in a desiccator and used without further purification. $SOCl_2$ was distilled before use and benzene dried over molecular sieves. *O*-(Trimethylsilyl)hydroxylamine was synthesized as described[15] but can be purchased from Fluka. Melting points were measured with a Büchi apparatus and are uncorrected. Microanalyses were obtained using a Perkin–Elmer 240 or 2400 element analyzer. ^{1}H-NMR/^{13}C-NMR spectra were obtained using a Bruker AMX 500 MHz NMR spectrometer, IR spectra using a Perkin–Elmer 580 spectrometer and UV spectra using a Perkin–Elmer Lambda 7 UV/VIS spectrophotometer. A solution of the title compounds in DMF was prepared (approximately 2×10^{-4} M in 2.5 mL DMF) and the UV spectra were recorded at approximately max. O.D. Then diisopropylethylamine (DIEA, 10 µL) was added to each sample and the recordings were repeated.

3,4-Dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (2a):
Anthranilic acid (**1a**; 10 mmol, 1.37 g) is suspended in benzene (20 mL) in a dried, N_2 filled round-bottomed flask fitted with a magnetic stirrer and condenser. $SOCl_2$ (40 mmol, 4.76 g) in benzene (10 mL) is added under stirring and the resultant mixture heated to reflux until a clear solution is obtained. Benzene and residual $SOCl_2$ is evaporated at 1–2 Torr and the residual sulfinamide anhydride resuspended in benzene (20 mL). *O*-(Trimethylsilyl)hydroxylamine (25 mmol, 2.63 g) in dry benzene (10 mL) is then added and the mixture stirred overnight (12 h) in an N_2 atmosphere. The solvent is evaporated and the resulting hydroxamic acid is suspended in a mixture of 12 N HCl (3 mL) and ice water (40 mL). A cold solution of $NaNO_2$ (22 mmol, 1.52 g) in H_2O (22 mL) is slowly added keeping the temperature below 4 °C. After 30 min the crude Dhbt-OH is filtered off and washed several times with cold H_2O and dried in a desiccator to constant weight; yield of yellow powder: 1.29 g (79%). 0.4 g of the crude product is extracted with 0.5 N NaOH (5 mL + 1 mL) and the basic extracts washed with CH_2Cl_2 (3 × 2 mL). 4 N HCl (1 mL) is added and the precipitated Dhbt-OH recrystallized from EtOH/H_2O to give colorless needles; yield: 0.27 g (68%) (Lit.[13] 28% calc. from methyl anthranilate); mp 186–187 °C (dec.) (Lit.[13] mp 182.0–182.5 °C).

$C_7H_5N_3O_2$	calc.	C 51.54	H 3.09	N 25.76	
(163.1)	found	51.65	3.10	25.59	

IR (KBr): ν = 3200–2500 (OH), 1670, 1635 cm^{-1} (C=O, N=N).
UV (DMF): λ_{max} = 302 nm (ε = 5700).
UV (DMF + Et_2NH): λ_{max} = 358 nm (ε = 7450), 430 nm (ε = 4100).
^{1}H-NMR (DMSO-d_6/TMS): δ = 2.51 (s, OH), 7.93 (dt, H_7), 8.06 (dt, H_6), 8.19 (dd, H_5), 8.26 (dd, H_8), J_{ortho} = 7.5 Hz, J_{meta} = 1 Hz.
^{13}C-NMR (DMSO-d_6/TMS): δ = 121.27 (C_{4a}), 124.77 (C_5), 128.11 (C_8), 132.49 (C_6), 135.09 (C_7), 143.89 (C_{8a}), 151.06 (C_4).

3,4-Dihydro-3-hydroxy-6-iodo-4-oxo-1,2,3-benzotriazine (2b):
As described for Dhbt-OH **(2a)** from 5-iodoanthanilic acid (**1b**; 5 mmol, 1.32 g) and $SOCl_2$ (20 mmol, 2.38 g), *O*-(trimethylsilyl)hydroxylamine (12.5 mmol, 1.32 g); yield of yellow powder: 0.99 g (69%). Recrystallization of the crude product (0.12 g) from 20% EtOH/H_2O gives light yellow needles; yield: 0.12 g (60%) (Lit.[1] yield 28% calc. from methyl 5-iodoanthranilate); mp 170–171 °C (dec) (Lit.[1] mp 178 °C (dec.)).

$C_7H_4IN_3O_2$	calc.	C 29.09	H 1.39	I 43.91	N 14.54
(289.0)	found	28.97	1.25	44.20	14.23

UV (DMF): λ_{max} = 318 nm (ε = 9200).
UV (DMF + Et_2NH): λ_{max} = 370 nm (ε = 8750), 446 nm (ε = 4000).
^{1}H-NMR (DMSO-d_6/TMS): δ = 2.50 (s, OH), 7.95 (d, H_8), 8.36 (d, H_7), 8.56 (s, H_5), J_{ortho} = 8.5 Hz.
^{13}C-NMR (DMSO-d_6/TMS): δ = 100.04 (C_6), 122.66 (C_{4a}), 129.61 (C_8), 133.07 (C_5), 143.01 (C_{8a}), 143.89 (C_7), 149.72 (C_4).

3,4-Dihydro-3-hydroxy-6-methyl-4-oxo-1,2,3-benzotriazine (2c):
As described for Dhbt-OH **(2a)** from 5-methylanthranilic acid (**1c**; 10 mmol, 1.51 g), $SOCl_2$ (40 mmol, 4.76 g) and *O*-(trimethylsilyl)hydroxylamine (25 mmol, 2.63 g); yield of yellow powder: 1.20 g (68%). The crude product (0.28 g) is extracted with 0.5 N NaOH (6 mL + 2 mL) and the basic extracts washed with CH_2Cl_2 (3 × 2 mL). 4 N HCl is added to pH ≈ 0 and the precipitated 6-methyl-Dhbt-OH **(2c)** recrystallized from EtOH/H_2O to give a colorless powder; yield: 0.27 g (54%); mp 202–203 °C (dec).

$C_8H_7N_3O_2$	calc.	C 54.24	H 3.98	N 23.72	
(177.2)	found	53.98	3.88	23.73	

IR (KBr): ν = 3200–2500 (OH), 1670, 1640 cm^{-1} (C=O, N=N).
UV (DMF): λ_{max} = 306 nm (ε = 6250).
UV (DMF + Et_2NH): λ_{max} = 362 nm (ε = 6450), 418 nm (ε = 3500).
^{1}H-NMR (DMSO-d_6/TMS): δ = 2.54 (s, OH, CH_3), 7.87 (dd, H_7), 8.05 (d, H_5), 8.08 (d, H_8), J_{ortho} = 8.5 Hz, J_{meta} = 2 Hz.
^{13}C-NMR (DMSO-d_6/TMS): δ = 21.31 (CH_3), 121.17 (C_{4a}), 123.89 (C_5), 128.00 (C_8), 136.36 (C_7), 142.26 (C_6), 143.40 (C_{8a}), 151.01 (C_4).

7-Chloro-3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (2d):
As described for Dhbt-OH **(2a)** from 4-chloroanthranilic acid (**1d**; 100 mmol, 17.15 g), $SOCl_2$ (400 mmol, 47.59 g) and *O*-(trimethylsilyl)hydroxylamine (250 mmol, 26.30 g); yield of yellow powder: 9.02 g (91%). The crude product (2.0 g) is continuously extracted with MeOH for 2 h. The solvent is evaporated, and the resultant yellow powder is recrystallized from H_2O; yield of slightly yellow needles: 1.14 g (57%); mp 176–178 °C (dec).

$C_7H_4ClN_3O_2$	calc.	C 42.55	H 2.04	Cl 17.94	N 21.27
(202.3)	found	42.80	1.77	18.29	21.18

UV (DMF): λ_{max} = 308 nm (ε = 5950).
UV (DMF + Et_2NH): λ_{max} = 366 nm (ε = 10050), 446 nm (ε = 4700).
^{1}H-NMR (DMSO-d_6/TMS): δ = 2.53 (s, OH), 7.95 (dd, H_6), 8.27 (d, H_5), 8.33 (d, H_8), J_{ortho} = 8.5 Hz, J_{meta} = 2 Hz.
^{13}C-NMR (DMSO-d_6/TMS): δ = 121 (C_{4a}), 127 (C_5), 129.39 (C_8), 132.70 (C_6), 138 (C_7), 143 (C_{8a}), 151 (C_4).

3,4-Dihydro-3-hydroxy-7-nitro-4-oxo-1,2,3-benzotriazine (2e):
As described for Dhbt-OH **(2a)** from 4-nitroanthanilic acid (**1e**; 50 mmol, 9.11 g), $SOCl_2$ (200 mmol, 23.79 g), *O*-(trimethylsilyl)hydroxylamine (125 mmol, 13.15 g); yield of yellow powder: 8.8 g (85%). The crude product (2.7 g) is continuously extracted with MeOH for 2 h, H_2O (100 mL) is added and the solvent is concentrated *in vacuo* to the beginning of precipitation. After cooling to −16 °C the yellow crystals are collected on a filter and washed with H_2O. **2e** is recrystallized from H_2O; yield of yellow needles: 2.13 g (79%); mp 163–164 °C (dec).

Alternatively the crude product (0.2 g) is extracted several times with Et_2O, the solvent is evaporated, and the yellow powder recrystallized from H_2O; yield of yellow needles: 0.11 g (55%); mp 165–166 °C (dec).

$C_7H_4N_4O_4 \cdot 0.5\,H_2O$	calc.	C 38.72	H 2.32	N 25.80	
(219.3)	found	38.56	2.30	25.83	

UV (DMF): λ_{max} = 306 nm (ε = 7100).
UV (DMF + Et_2NH): λ_{max} = 434 nm (ε = 9550), 540 nm (ε = 2350).
^{1}H-NMR (CD_3CN/TMS): δ = 3.62 (s, OH), 8.49 (d, H_5), 8.55 (dd, H_6), 8.95 (d, H_6), J_{ortho} = 9 Hz, J_{meta} = 2 Hz.

3,4-Dihydro-3-hydroxy-6,7-dimethyl-4-oxo-1,2,3-benzotriazine (2f): As described for Dhbt-OH **(2a)** from 4,5-dimethylanthranilic acid (**1f**; 10 mmol, 1.65 g), $SOCl_2$ (40 mmol, 4.76 g) *O*-(trimethylsilyl)-hydroxylamine (25 mmol, 2.63 g). Reflux of the anthranilic acid and $SOCl_2$ is carried out in a mixture of benzene (30 mL) and THF (20 mL). Yield of brown powder: 1.32 g (69%). The crude product (0.21 g) is extracted with 0.5 N NaOH (6 mL + 2 mL) and the basic extracts washed with CH_2Cl_2 (3 × 2 mL). 4 N HCl is added to pH ≈ 0 and **2f** precipitated as a colorless powder; yield: 0.14 g (67%); mp 195–197 °C (dec).

$C_9H_9N_3O_2$	calc.	C 56.54	H 4.74	N 21.98
(191.2)	found	56.43	4.79	21.46

UV (DMF): λ_{max} = 308 nm (ε = 7050).

UV (DMF + Et_2NH): λ_{max} = 356 nm (ε = 6600), 416 nm (ε = 3950).

^{1}H-NMR (DMSO-d_6/TMS): δ = 2.46, 2.48 (2 s, 2 x CH_3), 2.51 (s, OH), 7.97 (s, H_5), 8.01 (s, H_8).

^{13}C-NMR (DMSO-d_6/TMS): δ = 19.81 (CH_3), 19.90 (CH_3), 119.16 (C_{4a}), 124.17 (C_5), 127.76 (C_8), 142.74 (C_6), 143 (C_{8a}), 145.62 (C_7), 151 (C_4).

3,4-Dihydro-3-hydroxy-6,7-dimethoxy-4-oxo-1,2,3-benzotriazine (2g):
As described for Dhbt-OH **(2a)** from 4,5-dimethoxyanthranilic acid (**1g**; 20 mmol, 3.94 g), $SOCl_2$ (80 mmol, 9.52 g) *O*-(trimethylsilyl)hydroxylamine (50 mmol, 5.26 g); yield of brown powder: 2.89 g (65%). To the crude product (0.5 g) is added 2 N NaOH (5 mL) and the sodium salt of **2g** is isolated by centrifugation. The salt is washed with 0.5 N NaOH (1 mL) and with H_2O (1 mL). 4 N HCl (2 mL) is added and **2g** is recrystallized from EtOH/H_2O; yield of colorless needles: 0.32 g (64%); mp 248–251 °C (dec).

$C_9H_9N_3O_4$	calc.	C 48.43	H 4.06	N 18.83
(223.2)	found	48.61	4.12	18.92

IR (KBr): ν = 3200–2500 (OH), 1655, 1605 cm^{-1} (C=O, N=N).

UV (DMF): λ_{max} = 320 nm (ε = 5850).

UV (DMF + Et_2NH): λ_{max} = 350 nm (ε = 3650), 404 nm (ε = 3050).

^{1}H-NMR (DMSO-d_6/TMS): δ = 3.34 (s, OH), 3.98 (s, CH_3O), 3.99 (s, CH_3O), 7.54 (s, H_5), 7.63 (s, H_8).

^{13}C-NMR (DMSO-d_6/TMS): δ = 56.48 (CH_3O), 56.55 (CH_3O), 103.13 (C_5), 108.00 (C_8), 115.83 (C_{4a}), 140.58 (C_{8a}), 150.67 (C_4), 153.00 (C_6), 154.77 (C_7).

3,4-Dihydro-3-hydroxy-4-oxonaphtho[2,3-*d*]-1,2,3-triazine (2h):
The starting 3-amino-2-naphthoic acid (Aldrich, 80%) is purified before use. 3-Amino-2-naphthoic acid (**1h**; 5 g) is boiled with 4 N HCl (150 mL) and the hot solution filtered. The filter cake is boiled with 2 N HCl (100 mL) and filtered as before. On cooling the hydrochloride precipitates from the combined filtrates, which is collected on a filter, and washed with cold H_2O. The filter cake is suspended in H_2O (40 mL), conc. NaOH is added adjusting pH to ≈ 12 and the suspension heated to 85 °C. The solution is filtered and 12 N HCl added to pH ≈ 3 and adjusted to 4.5 with 10% NaOAc. The yellow precipitate is collected on a filter and dried to constant weight; yield 3.75 g (75%); mp 213–214 °C (dec) (mp Lit.[14] 216–217 °C). 3,4-Dihydro-3-hydroxy-4-oxonaphtho[2,3-*d*]-1,2,3-triazine **(2h)** is synthesized as described for Dhbt-OH **(2a)** from the purified 3-amino-2-naphthoic acid (**1h**, 10 mmol, 1.87 g), $SOCl_2$ (40 mmol, 4.76 g) and *O*-(trimethylsilyl)hydroxylamine (25 mmol, 2.63 g); yield of brown powder: 1.50 g (70%). The crude product (0.3 g) is recrystallized from 75% EtOH/H_2O, and then three times from 50% EtOH/H_2O each time adding a little active carbon to the warm solution before filtering; yield of yellow powder: 72 mg (36%); mp 195–196 °C (dec).

$C_{11}H_7N_3O_2$	calc.	C 61.97	H 3.31	N 19.71
(213.2)	found	61.57	3.30	19.03

IR (KBr): ν = 3200–2500 (OH), 1675, 1650 cm^{-1} (C=O, N=N).

UV (DMF): λ_{max} = 356 nm (ε = 6200).

UV (DMF + Et_2NH) λ_{max} = 364 nm (ε = 4900), 380 nm (ε = 5850), 398 nm (ε = 5100), 504 nm (ε = 1400).

^{1}H-NMR (DMSO-d_6/TMS): δ = 7.84 (m, H_6, H_7), 8.40 (dt, 2 H, H_5, H_8), 8.92 and 9.02 (2 s, H_9, H_{10}), 12.62 (s, OH).

^{13}C-NMR (DMSO-d_6/TMS): δ = 118.69 (C_{4a}), 126.14 (C_{10}), 128.33 (C_9), 129.07, 129.17, 129.23, 129.33 (C_5-C_8), 133.55, 135.37 (C_{8a}, C_{10a}), 140.04 (C_{9a}), 151.34 (C_4).

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