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# A NEW PEPTIDE SYNTHESIS USING 3-(SUCCINIMIDOXY)-1,2-BENZOISOTHIAZOLE-1,1-DIOXIDE. APPLICATION TO SYNTHESIS OF LEUCINE-ENKEPHALIN

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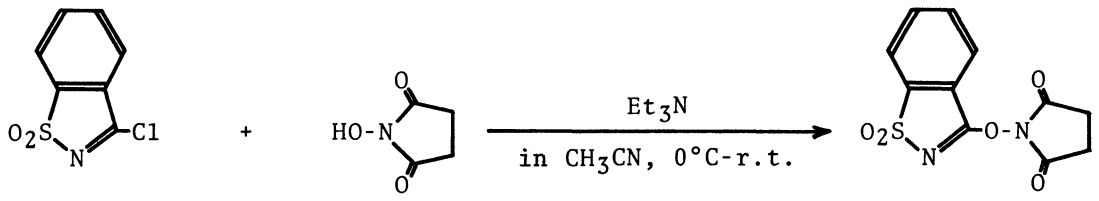
3-(Succinimidoxy)-1,2-benzoisothiazole-1,1-dioxide was found to be a useful reagent to convert the carboxylic acids to the corresponding N-hydroxysuccinimide esters. Various dipeptides and leucine-enkephalin were prepared using the reagent in good yields.

Use of 1,2-benzoisothiazol-3(2H)-one-1,1-dioxide (sacchalin) derivatives for peptide synthesis was first reported by Micheel and Lorenz in 1963,[1] and then Hettler found that 3-chloro-1,2-benzoisothiazole-1,1-dioxide (I) is effective for the esterification of carboxylic acids in DMSO.[2] In connection with our recent study on the condensing reagents,[3] we were interested in such saccharin derivatives because of their generally good crystalline property.[2]

It is well known that N-hydroxysuccinimide (II) is a useful reagent for the preparation of activated esters and effective to suppress racemization in peptide synthesis, so we tried to prepare 3-(succinimidoxy)-1,2-benzoisothiazole-1,1-dioxide (BID-OSu) as follows: to a mixed solution of I (687 mg, 3.4 mmol) and II (430 mg, 3.7 mmol)

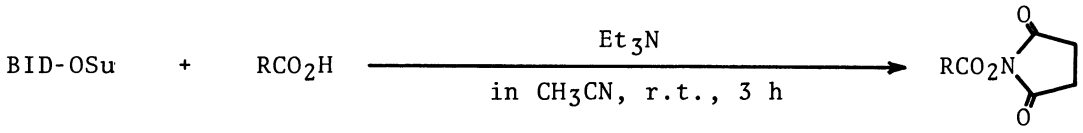



I II BID-OSu, 83%

in acetonitrile (20 ml) was added triethylamine (344 mg, 3.4 mmol) at 0°C. A white crystalline precipitate started to separate almost immediately. After stirring overnight at room temperature, the precipitate was filtered off and recrystallized from acetonitrile to give pure BID-OSu in an 83% yield (798 mg). Mp 278-280°C. Found: C,

47.30; H, 2.80; N, 9.88%. Calcd for $C_{11}H_8N_2O_5S$: C, 47.15; H, 2.88; N, 10.00%. This compound is quite stable on storage, whereas I is very unstable.

BID-OSu thus prepared was allowed to react with benzoic acid to check its reactivity. To a mixed suspension of BID-OSu (140 mg, 0.5 mmol) and benzoic acid (61 mg, 0.5 mmol) in acetonitrile (3 ml) was added a solution of triethylamine (51 mg, 0.5 mmol) in acetonitrile (2 ml) at room temperature under nitrogen. The suspension became clear after a few minutes because of the consumption of slightly soluble BID-OSu in progress of reaction. After stirring for 3 h, the solvent was evaporated in vacuo and the resulting residue was subjected to preparative TLC (silica gel, ethyl acetate/benzene=1/5) to give the N-benzoyloxysuccinimide (III) in an 87% yield (95 mg, mp 139-140°C).[3b] In a similar manner, Boc-Ala-OSu (IV) was obtained from Boc-Ala-OH and BID-OSu in an 80% yield (mp 164-165°C, $[\alpha]_D^{30}$ -50.4° (c 2.34, dioxane)).[4]




III (R=$C_6H_5$), 87%
IV (R=Boc-NHCHCH$_3$), 80%

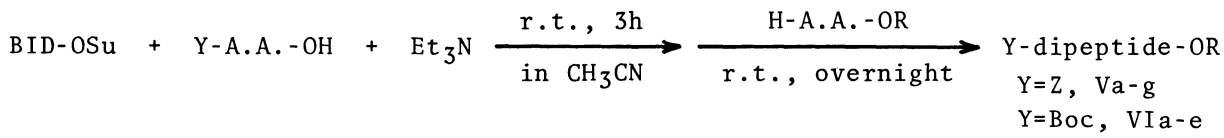
Since this result suggested that BID-OSu is useful to convert the carboxylic acids to the corresponding N-hydroxysuccinimide esters, the application of BID-OSu to peptide synthesis was next examined.

Z-Ile-OH (133 mg, 0.5 mmol) was allowed to react with BID-OSu (140 mg, 0.5 mmol) and triethylamine (51 mg, 0.5 mmol) under similar conditions mentioned above to convert to the activated ester (Z-Ile-OSu). Without isolation of the ester, HCl·H-Gly-OEt (77mg, 0.55 mmol) and triethylamine (56 mg, 0.55 mmol, in 3 ml acetonitrile) were added subsequently to the reaction mixture at room temperature. After stirring overnight, the solvent was evaporated and the residue was redissolved in ethyl acetate, and washed successively with 1 M-HCl, saturated sodium hydrogencarbonate and saturated sodium chloride. The residue obtained by evaporation of the solvent was subjected to preparative TLC (silica gel, ethyl acetate/benzene=1/1) to give the corresponding dipeptide (Z-Ile-Gly-OEt, Va) in an 85% yield (148 mg, $[\alpha]_D^{21}$ -25.2° (c 0.42, EtOH), mp 153-154°C).

In a similar way, other N-benzyloxycarbonyl dipeptide esters (Vb-g) and N-t-butoxycarbonyl dipeptide esters (VIa-e) were prepared in good yields without racemization as shown in Table.

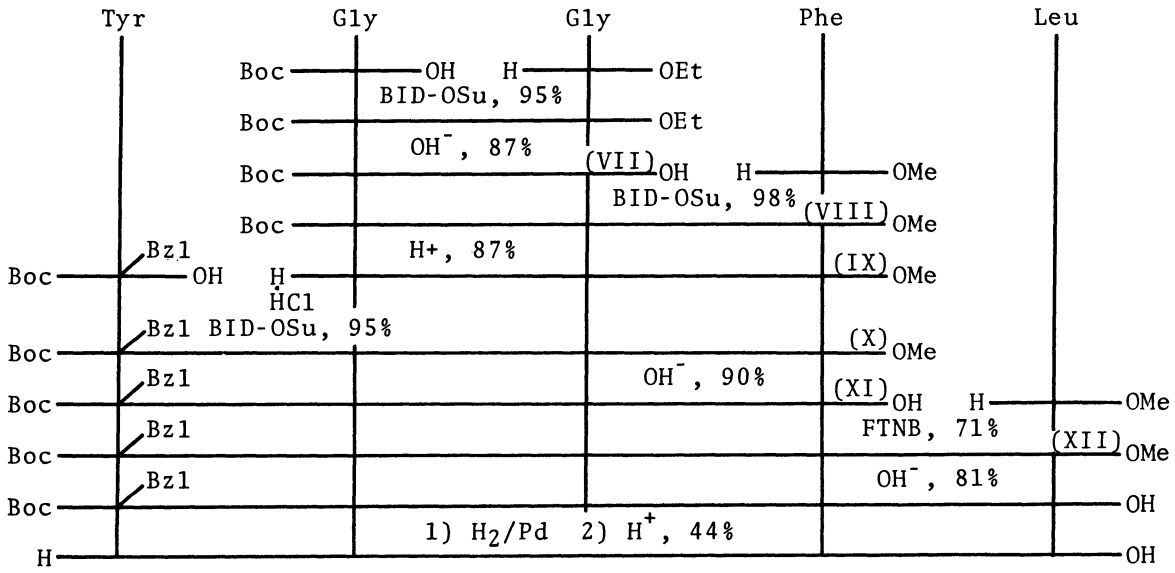
These satisfactory results encouraged us to apply the reagent to the synthesis

Table. Preparation of Protected Dipeptide Esters




| Product | Yield (%) | Mp (°C) (lit) | $[\alpha]_D$ (c, solv) (lit) | Ref. |
|---|---|---|---|---|
| Va, Z-Ile-Gly-OEt | 85 | 153-154 (155-156) | -25.2 (0.42, EtOH) (-25.6) | 5 |
| Vb, Z-Gly-Gly-OEt | 88 | 79-80 (80) | — | 6 |
| Vc, Z-Ala-Gly-OEt | 88 | 98-100 (99-100) | -22.1 (3.07, EtOH) (-22.2) | 5 |
| Vd, Z-Val-Gly-OEt | 81 | 164-165 (162-164) | -27.3 (1.37, EtOH) (-27.0) | 5 |
| Ve, Z-Met-Gly-OEt | 90 | 94-95 (94-96) | -19.5 (3.55, EtOH) (-19.8) | 7 |
| Vf, Z-Cys(Bzl)-Gly-OEt | 85 | 97-98 (97-99) | -28.5 (5.22, AcOEt) (-27.0) | 7 |
| Vg, Z-Ser-Thr-OEt | 72 | 125-126 (126-127) | +7.5 (2.13, DMF) (+6.9) | 8 |
| VIa, Boc-Phe-Val-OMe | 84 | 115-117 (118-119) | -10.9 (2.01, DMF) (-11.6) | 9 |
| VIb, Boc-Leu-Leu-OMe | 92 | 139-140 (141-142) | -49.1 (0.36, MeOH) (-50.4) | 10 |
| VIc, Boc-Ile-Ile-OMe | 75 | 156-157 (158-159) | -38.0 (0.25, MeOH) (-33.3) | 11 |
| VId, Boc-Ala-Ala-OMe | 85 | 107-109 (105-108) | -63.1 (1.28, MeOH) (-63.8) | 9 |
| VIe, Boc-Ala-Val-OMe | 79 | 67-69 (63-64) | -50.4 (0.39, MeOH) (-49.5) | 3c |

Scheme. Synthesis of Leucine-Enkephalin



of a biologically active peptide. The synthesis of leucine-enkephalin with opiate-agonist activity was carried out according to the above Scheme. Boc-Gly-Gly-OEt derived from Boc-Gly-OH and H-Gly-OEt by BID-OSu method in a 95% yield was saponified to give VII (87%, mp 132-133°C), which was condensed with H-Phe-OMe employing BID-OSu to afford the protected ester (VIII) in a 98% yield. After the deprotection of t-butoxycarbonyl group of VIII with 5 M-HCl in ethyl acetate, resulting IX (87%, mp 180-181°C, $[\alpha]_D^{18.8}$ +31.5° (c 1.17, AcOH)) was condensed with α-t-butoxycarbonyl-O-benzyl tyrosine by the use of BID-OSu to give the tetrapeptide ester (X, mp 112-113°C, $[\alpha]_D^{20}$ +13.6° (c 1.03, MeOH)) in a 95% yield. The last condensation step to form the pentapeptide ester (XII, mp 153-154°C, $[\alpha]_D^{22}$ -11.4° (c 1.74, MeOH)) from the deprotected tetrapeptide (XI, 90%, mp 88-90°C, $[\alpha]_D^{21.5}$ +18.2° (c 0.55, MeOH)) and H-Leu-OMe was accomplished by FTNB (2-fluoro-1,3,5-trinitrobenzene) method.[3c, 12] A saponification of XII followed by hydrogenation over 10% Pd-C and the subsequent treatment with 5 M-HCl in dioxane gave leucine-enkephalin as reported previously.[3c]

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