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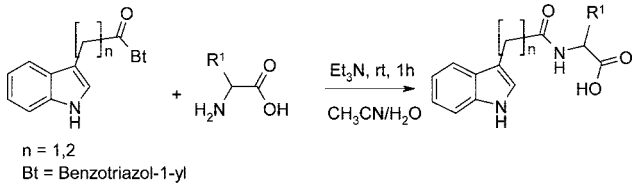
Syntheses of IAA- and IPA-Amino Acid Conjugates

Alan R. Katritzky,* Levan Khelashvili, and Munawar Ali Munawar

Center for Heterocyclic Compounds, Department of Chemistry, University of Florida, Gainesville, Florida 32611-7200

katritzky@chem.ufl.edu

Received August 13, 2008



Amino acid derivatives of IAA and IPA are prepared conveniently and efficiently by coupling of readily available **2a-b** with diverse free amino acids **3a-g** and **(3c+3c′)** to give compounds **4a-j**, **(4c+4c′)** and **(4h+4h′)** in 38−70% yields. Similarly, **2a-b** afforded IAA and IPA peptide conjugates **6a-b** in 32−40% yields. Complete retention of chirality was supported by NMR and HPLC analysis.

Indole-3-acetic acid (IAA) is an indispensable plant hormone (auxin), which also occurs naturally as numerous 'conjugates' linked to amino acid, sugar, or inositol residues.[1] Gene expression, cell division, cell elongation and differentiation in plant tissue are all regulated by indole-3-acetic acid auxins,[1,2] which can also control vascularization, phototropism, geotropism, fruit development, flower development, and apical dominance.[3] IAA is a major metabolite of tryptophan in animals, being formed in body tissues and by intestinal bacteria.[4,5]

Many plant species convert indole-3-acetic acid (IAA) into ether-insoluble metabolites, including the amino acid conjugate, indole-3-acetylaspartic acid (IAA-Asp), as identified by chromatography, color tests, and biological activity.[6] Indole-3-acetylysine (IAA-Lys) was isolated from *Pseudomonas savastanoi*.[7]

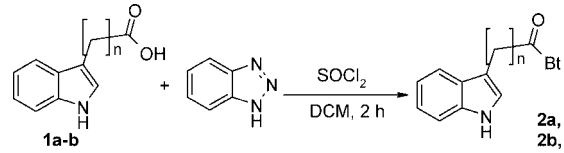
Indole-3-propionic acid (IPA) is endogenous plant hormone and its amino acid conjugates are known to interact with serum albumin.[8]

Numerous literature approaches to 3-indoleacetylated amino acids utilize: (i) mixed-anhydrides,[8−11] (ii) active esters,[12−16] (iii) acid chlorides,[17] and (iv) DCC as a coupling reagent.[18−21] Hydroxybenzotriazole (HOBt) has been used to facilitate acylations but is explosive and can no longer be purchased or shipped.

N-Acylbenzotriazoles[22] are easily prepared, chirally stable reagents for *N*[23]-, *C*[24]- and *O*-acylation.[25] *N*-(Aminoacyl)benzotriazoles prepared from *N*-protected alpha-amino acids have been successfully utilized for synthesis of di-, tri-, and tetrapeptides.[26]

I. Preparation of Benzotriazole Derivatives of IAA and IPA

1-(1*H*-Benzotriazol-1-yl)-2-(1*H*-indol-3-yl)ethanone **2a** and 1-(1*H*-benzotriazol-1-yl)-3-(1*H*-indol-3-yl)propan-1-one **2b** were prepared by a standard method (eq 1).[26a] Treatment of indole-3-acetic acid **1a** or indole-3-propionic acid **1b** with 4 equiv BtH and 1 equiv $SOCl_2$ at room temperature for 2 h gave products **2a**−**b** in 86−90% yield. Compounds **2a**−**b** are stable indefinitely at 20 °C.



(1)

II. Preparation of Amino Acid Derivatives of Indole-3-acetic Acid and Indole-3-propionic Acid. IAA-Bt **2a** was

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10.1021/jo8017796 CCC: $40.75 © 2008 American Chemical Society
Published on Web 10/22/2008

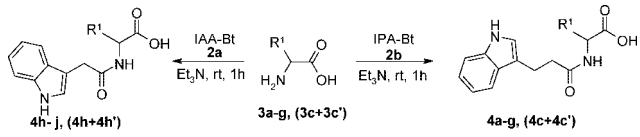
TABLE 1. Preparation of IAA and IPA Amino Acid Conjugates

					previous work		
entry	reactant	product	yield (%)[a]	mp (°C)	yield (%)	mp (°C)	lit.
1	Gly **3a**	IAA-Gly-OH **4a**	40	87−88	40	86−87	ref 10
2	L-Ala **3b**	IAA-L-Ala-OH **4b**	63	138−140	not reported	not reported	ref 9
3	L-Phe **3c**	IAA-L-Phe-OH **4c**[b]	66	148−150	not reported	174−176	ref 13
4	D,L-Phe **(3c+3c′)**	IAA-D,L-Phe-OH **(4c+4c′)**[c]	70	198−200	not reported	not reported	ref 13
5	L-Trp **3d**	IAA-L-Trp-OH **4d**	45	181−182	9	181−183	ref 17
6	L-Met **3e**	IAA-L-Met-OH **4e**	40	123−124	not reported	not reported	ref 20
7	L-Arg **3f**	IAA-L-Arg-OH **4f**	45	174−176	failed	not reported	ref 20
8	L-Lys **3g**	IAA-L-ω-Lys-OH **4g**	50	241−242	57	240−245	ref 19
9	L-Phe **3c**	IPA-L-Phe-OH **4h**[d]	45	160−162	−	−	−
10	D,L-Phe **(3c+3c′)**	IPA-D,L-Phe-OH **(4h+4h′)**[e]	53	159−160	not reported	155−156	ref 27
11	L-Trp **3d**	IPA-L-Trp-OH **4i**	38	230−231	−	−	−
12	L-Arg **3f**	IPA-L-Arg-OH **4j**	50	180−181	−	−	−
13	Gly-D-Phe **5**	IAA-Gly-D-Phe **6a**	32	132−134	−	−	−
14	Gly-D-Phe **5**	IPA-Gly-D-Phe **6b**[f]	42	216−218	−	−	−

[a] isolated yield. [b] Retention time for **4c** = 9.02 min. [c] Retention time for **(4c+4c′)** = 9.16 and 9.68 min. [d] Retention time for **4h** = 7.02 min. [e] Retention time for **(4h+4h′)** = 6.67 and 8.80 min. [f] Retention time for **6a** = 10.91 min.

coupled with diverse free amino acids **3a−g** and **(3c+3c′)** in the presence of Et_3N (2 equiv.) in aqueous acetonitrile at RT during 1 h. (Compound numbers written within brackets represent a diastereomeric mixtures or racemates; compound numbers without brackets represent enantiomers.) Work up afforded **4a-g** and **(4c+4c′)** in 40−70% yield (Scheme 1 and Table 1).

SCHEME 1

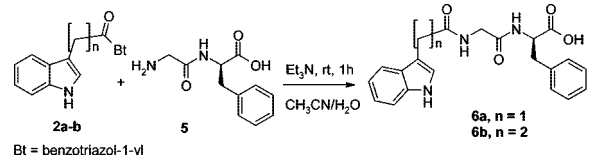


Compounds **4h−j** and **(4h+4h′)** were obtained similarly by coupling benzotriazole activated indole-3-propionic acid **2b** with free amino acids **3c−d,f** and **(3c+3c′)** in 38−53% yield (Scheme 1 and Table 1). All products **4a−j**, **(4c+4c′)** and **(4h+4h′)** were characterized by ^1H NMR, ^{13}C NMR and elemental analysis.

Amino acids are known to racemize under harsh reaction conditions. The HPLC (detection at 220 nm, flow rate 0.5 mL/min, and MeOH/H_2O (50:50) as solvent) results showed single peaks for **4c** and **4h**. By contrast two peaks were observed for the corresponding racemic mixtures **(4c+4c′)** and **(4h+4h′)**confirming the enantiopurity of IAA-L-Phe-OH **4c** and IPA-L-Phe-OH **4h**.

As a further application of this synthetic approach, **2a−b** were coupled with free dipeptide Gly-D-Phe-OH **5** to give compounds **6a-b** in 32−42% yield (eq 2, Table 1). HPLC analysis confirmed the enantiopurity of **6a** showing single peak at 10.91 min.

In summary, the convenient preparation of IAA and IPA conjugates using stable benzotriazole activated acids has been



(2)

demonstrated under mild reaction conditions. The products were obtained in yields of 32−70% (average 49%) by simple isolation and purification procedures. All reactions were carried out at 20 °C and the products were isolated by washing with 6N HCl, whereas literature methods use low temperatures (0−5 °C, −8 °C) and include complex procedures[10,12,14,17] The reactions need 1 h to complete in contrast to literature methods[13] (up to 28 h) and give higher or comparable yields (Table 1) Our method gives enanthiopure products, and is applicable to a variety of amino acids, whereas some literature methods are restricted.[20] We believe that our methodology should work for IAA-Asp as well, although the isolation might be difficult because of the free carboxylic groups in this molecule. Finally the method allows the use of free amino acids as coupling components, does not require anhydrous conditions and is cost-effective.

Thus the advantages using Bt-activation over existed methods are: (a) comparable or better yields, (b) simple preparative and purification procedures, (c) short reaction time, (d) proven enantiopurity, (e) applicability to a variety of amino acids, (f) low cost, and (g) use of aqueous conditions.

Experimental Section

General Procedure for the Preparation of 4a−e,h−i, (4c+4c′) (4h+4h′). Quantities of **2a−b** (0.5 mmol) were added at 20 °C to a solution of α-amino acid (0.5 mmol) **3a−e,g, (3c+3c′)** in CH_3CN (7 mL)/H_2O (3 mL) in the presence of Et_3N (1 mmol). The reaction mixture was stirred at 20 °C until the starting material was completely consumed as observed by TLC using hexane/ethyl acetate (2:1) as the solvent. After 1 mL of 6 N HCl was added, the solution was concentrated under reduced pressure to remove acetonitrile and the residue was extracted with EtOAc (20 mL). The organic extract was washed with 4N HCl (3 × 5 mL) and sat. NaCl (10 mL), and then dried (anhydrous $MgSO_4$). Evaporation of the solvent gave the desired product in pure form, which was further recrystallized from CH_2Cl_2/hexane unless otherwise specified.

(*S*)-2-(3-(1H-Indol-3-yl)propanamido)-3-(1H-indol-3-yl)propanoate (IPA-L-Trp-OH, 4i). White microcrystals (38%); mp 230−231 °C, ^1H NMR (DMSO-d_6): δ 2.47−2.54 (m, 2H),

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2.82−2.96 (m, 2H), 3.00−3.09 (m, 1H), 3.19 (dd, J = 14.4, 4.9 Hz, 1H), 4.49−4.62 (m, 1H), 6.95−7.08 (m, 2H), 7.03−7.20 (m, 4H), 7.30−7.40 (m, 2H), 7.51−7.63 (m, 2H), 8.25 (d, J = 7.7 Hz, 1H), 10.78 (s, 1H), 10.88 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 20.9, 27.2, 36.0, 53.0, 110.0, 111.3, 111.4, 113.8, 118.1, 118.2, 118.4, 120.9, 122.1, 123.6, 127.0, 127.3, 136.1, 136.2, 171.9, 173.6. Anal. calcd for $C_{22}H_{21}N_3O_3$: C, 70.38; H, 5.64; N, 11.19. Found: C, 70.08; H, 5.68; N, 11.32.

(*R*)-2-(2-(2-(1H-Indol-3-yl)acetamido)acetamido)-3-phenylpropanoate (IAA-Gly-D-Phe-OH, 6a). White microcrystals (32%); mp 132−134 °C, ^{1}H NMR (DMSO-d_6): δ 2.83 (dd, J = 13.5, 8.5 Hz, 1H), 3.02 (dd, J = 13.7, 5.1 Hz, 1H), 3.54 (s, 2H), 3.60−3.85 (m, 2H), 4.38−4.48 (m, 1H), 6.91−7.01 (m, 1H), 7.02−7.10 (m, 1H), 7.16−7.30 (m, 6H), 7.33 (d, J = 8.1 Hz, 1H), 7.54 (d, J = 8.0 Hz, 1H), 8.03 (t, J = 5.9 Hz, 1H), 8.14 (d, J = 8.1 Hz, 1H), 10.88 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 32.4, 36.8, 41.7, 53.4, 108.6, 111.3, 118.3, 118.7, 120.9, 123.9, 126.5, 127.2, 128.2, 129.1, 136.1, 137.4, 168.9, 171.0, 172.8. Anal. calcd for $C_{21}H_{21}N_3O_4$: C, 66.48; H, 5.58; N, 11.07. Found: C, 66.17; H, 5.33; N, 10.84.

Acknowledgment. We thank Higher Education Commission of Pakistan for financial support of M.A.M.

Supporting Information Available: Experimental details and spectroscopic data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO8017796