Int. J. Peptide Protein Res. **32**, 1988, 279–291

Synthesis of functionalized non-natural amino acid derivatives via amidoalkylation transformations

PHILIPPE LEGALL*, KAILASH N. SAWHNEY, JUDITH D. CONLEY and HAROLD KOHN

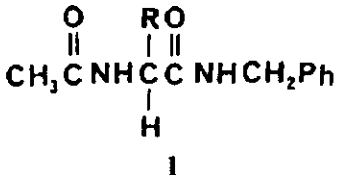
Department of Chemistry, University of Houston, Houston, TX, USA

Received 16 February, accepted for publication 2 May 1988

Synthetic routes have been developed for the preparation of functionalized amino acid derivatives in which the α-substituent at carbon 2 is either an aromatic or a heteroaromatic group. The α-substituent was introduced using an amidoalkylation reaction using boron trifluoride etherate and proceeded in moderate yield with excellent regioselectivity. This protocol permitted the employment of the acid sensitive heterocycles: pyrrole, benzofuran, and indole. The scope and limitations of this procedure have been evaluated.

Key words: α-substituted; amido alkylation transformations; aromatic; heteroaromatic; non-natural amino acids

Recent studies conducted in our laboratory have drawn attention to the importance of α-functionalized derivatives of *N*-acetylglycine-*N*-benzylamide (**1**, R = H) as potential drug candidates for the treatment of epilepsy (1). In an effort to delineate the structure activity relationship of this novel class of anticonvulsants, select derivatives of **1** were required in which the α-substituent R was either an aromatic or a heteroaromatic moiety. Unfortunately, relatively few methods exist for the preparation of the corresponding free amino acids** thereby diminishing the likelihood of employing these substrates as starting materials for the synthesis of **1**. In this paper, we describe the use and limitations of amidoalkylation transformations*** for the preparation of functionalized derivatives of amino acids in which the R substituent is an aromatic moiety.



1

RESULTS AND DISCUSSION

Two different strategies (Scheme 1, Methods A and B) were investigated for the preparation of **1**. The approaches differ primarily in the sequence of reactions employed for the synthesis of the desired compound **1**. In

*Abstracted from the Masters dissertation of this author. Additional structure proof and experimental and spectra data may be found in this reference.

**The 2- and 3-thienyl compounds are commercially available (Aldrich Chemical Company). For leading references for procedures for the preparation of non-natural amino acides and related studies, see ref. 2.

*** For excellent discussions of this reaction, see ref. 3.

Method A, the initial target was the 2-substituted alkyl 2-acetamidoacetate **6**. Our synthesis of this compound was patterned after the procedure described by Ben-Ishai, Sataty & Bernstein for the preparation of methyl *N*-benzyloxycarbonyl-α-furanglycinate (2). Reaction of acetamide (**2**) with glyoxylic acid (**3**) yielded **4** in near quantitative yield (**4**), which upon dissolution in either methanol or ethanol and acid gave the corresponding alkyl 2-acetamido-2-alkoxyacetates **5a** and **5b**. Treatment of **5** with either furan or pyrrole in the presence of boron trifluoride etherate gave the α-substituted product **6** in moderate yield (51–62%). In the case of furan, only **6a** was observed in which substitution had occurred at the 2-position of the aromatic ring. Correspondingly, with pyrrole a 3.4:1 binary mixture of the aromatic 2(**6c**$_1$)- and 3(**6c**$_2$)- substituted compounds, respectively, was obtained. Unfortunately, attempts to convert **6** to the corresponding benzyl amide adduct **1** proved unsatisfactory. Low yields were obtained for the condensation of benzylamine with **6**. Similarly, unacceptable overall yields for **1** were experienced for the sequential conversion of **6** to the acid **7** (KOH, H_2O), followed by the coupling of the *N*-protected amino acid **7** with benzylamine (i.e., $ClCO_2R$, Et_3N; DCC)[*].

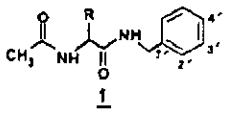
This synthetic obstacle was expeditiously circumvented by the use of the second pathway (Method B) outlined in Scheme 1. In this procedure, the coupling reaction was conducted prior to the amidoalkylation transformation. Treatment of alkyl 2-acetamido-2-alkoxyacetates **5a** and **5b** with benzylamine in alcoholic solution produced the corresponding 2-acetamido-*N*-benzyl-2-alkoxyacetamides **8a** and **8b**, respectively. Higher yields and cleaner product mixtures were noted for the synthesis of ethoxy adduct **8b** versus the methoxy derivative **8a**. Compound **8b** was converted to **1** by treatment with the appropriate aromatic or heteroaromatic substrate and boron trifluoride etherate. This synthetic route permitted the preparation of compounds **1a-1l**. Moderate yields (28–94%) for this step were observed for furan, 2-methylfuran, pyrrole, 1-methylpyrrole, thiophene, benzofuran, indole, phenol, *p*-cresol, anisole and thiophenol, while only a 4% yield was obtained for benzo[b]thiophene (Table 1). Employment of pyrazole, imidazole, pyridine, 3- and 4-hydroxypyridine, benzene, naphthalene, and *N*-acetylaniline as the aromatic substrate in this procedure led to no detectable product formation. No significant effort was made to vary either the acid or the solvent employed in the amidoalkylation step in order to improve the efficiency of this transformation.

Several interesting observations were noted concerning the conversion of the 2-ethoxy derivative **8b** to **1**. First, the employed conditions (boron trifluoride etherate, ether) permitted the use of the acid sensitive heterocycles: pyrrole, benzofuran, and indole. These substrates have found limited use in previous amidoalkylation transformations. (Zaugg (3), 5, 6). Second, in the reaction of pyrrole only a trace amount of the 3-substituted pyrrole product was detected (t.l.c. analysis). A much larger percentage of the corresponding adduct was observed when ester **5b** was employed as the starting material (Scheme 1, Method A). The high regioselectivity witnessed in the former transformation was also mirrored in the other reactions with heteroaromatic substrates. Typically, only one isomer was observed. This result was particularly surprising in the reactions involving benzofuran and benzo[b]thiophene. With benzofuran only the 2-substituted aromatic derivative was observed despite the known tendency of this heterocycle to undergo alkylation at both the 2- and 3-positions (7), while with benzo[b]thiophene none of the expected 3-substituted benzo[b]thiophene product was observed (7) but rather only a 4% yield of the 2-substituted adduct was isolated along with unreacted starting material. Third, all four substituted benzene substrates (phenol, *p*-cresol, anisole, and thiophenol) reacted to give a single product (t.l.c. analysis). In the case of phenol and anisole

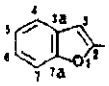
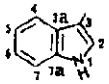
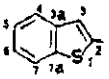
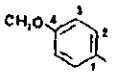
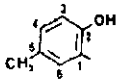
[*] The direct conversion of **4** to **7** was briefly examined (4). Addition of either furan or benzofuran to **4** in the presence of Lewis acids yielded the corresponding α-substituted amino acid derivatives in low yields (14–20%). For additional details, see footnote*

TABLE 1

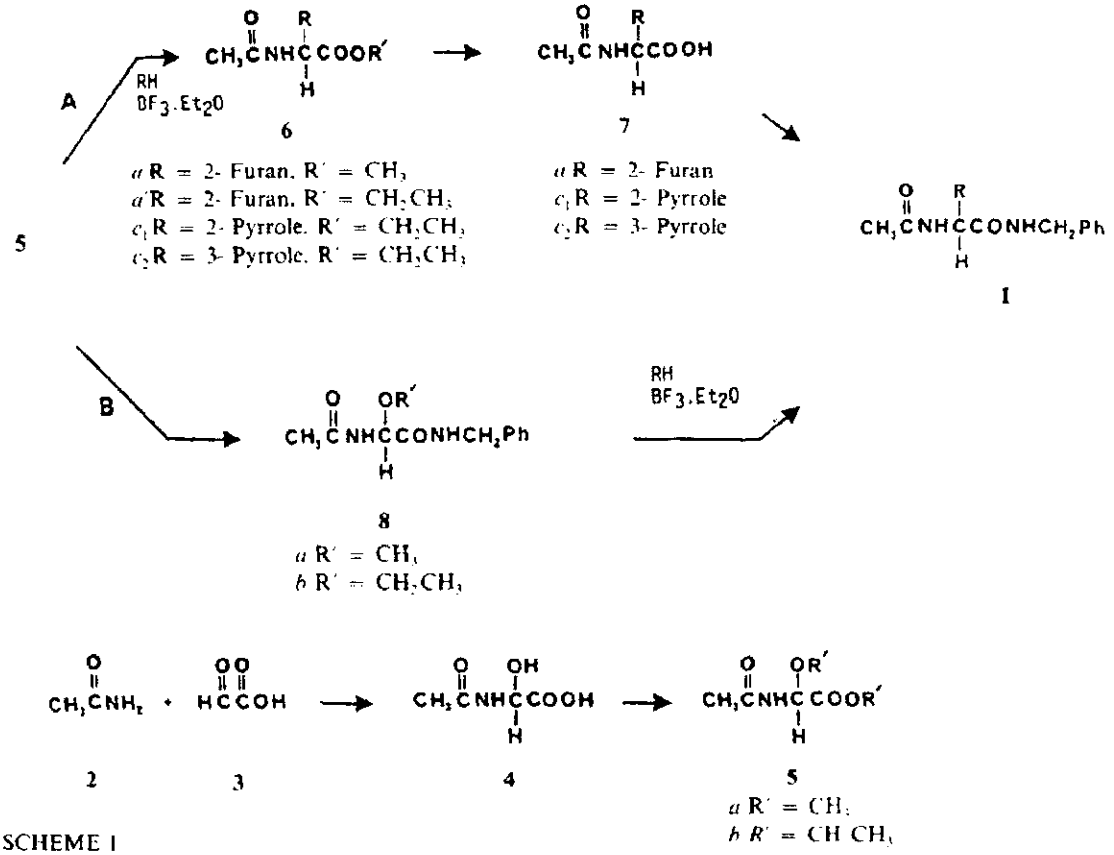
Selected physical and spectral data for 2-acetamido-N-benzyl-2-substituted acetamides (1)



1

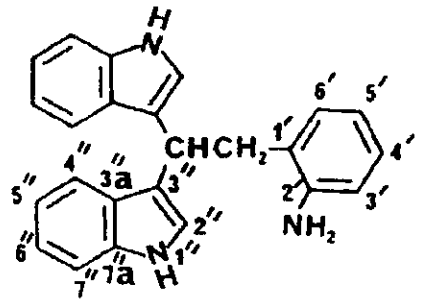
No.	R	Yield[a]	M.p.[b]	M^+/e[c]	^1H n.m.r.[d] α-CH	^{13}C n.m.r.[e] α-C
1a		58	178–179	273 (1)[f]	5.50 (d, 7.9)	50.95
1b		61	148–150	286 (3)	5.49 (d, 8.0)	53.23
1c[1]		35	174–175	271 (12)	5.42 (d, 6.9)[g]	52.65[g]
1d		62	179–181	285 (17)	5.52 (d, 7.8)	49.20
1e		37	167–169	289 (2)[f]	5.74 (d, 7.9)	52.20
1f		33	195–196	322 (5)	5.77 (d, 8.1)	51.22
1g		28	213–214	321 (5)	5.72 (d, 7.2)	49.98
1h		4	226–227	338 (8)	5.86 (d, 8.1)	52.70
1i		56	232–235	299 (1)[f]	5.34 (d, 7.4)	55.90
1j		62	196–198	313 (2)[f]	5.42 (d, 7.8)	55.78
1k		67	183–185	313 (7)[f]	5.63 (d, 7.6)	51.54
1l		94	165 167	315 (1)[f]	5.90 (d, 9.0)	57.65

[a] Purified yields (%) from 2-acetamido-*N*-benzyl-2-ethoxyacetamide (**8b**). [b] Melting points (°C) are uncorrected. [c] The molecular ion peak in the mass spectrum was obtained at an ionizing voltage of 70 ev. The number in the parentheses indicates the relative intensity of this ion relative to the base peak in the spectrum. [d] The 300 MHz ^1H n.m.r. spectra were taken in DMSO-d_6 unless otherwise indicated. The number in each entry is the chemical shift value (δ) observed in parts per million relative to TMS. The information in parentheses is the multiplicity of the signal, followed by the coupling constant (J) in Hertz. [e] The 75 MHz ^{13}C n.m.r. spectra were taken in DMSO-d_6 unless otherwise indicated. The number in each entry is the chemical shift value in parts per million relative to TMS. [f] The M + 1 peak was observed (McLafferty, F.W. "Interpretation of Mass Spectra," 2nd edn., W.A. Benjamin: Reading, MA, 1973). [g] N.m.r. spectrum was taken in CD_3CN.



SCHEME 1

the *para*-substituted adducts **1i** and **1j**, respectively, were observed, while with *p*-cresol only **1k** was isolated in which reaction had occurred *ortho*- to the phenolic group. Finally, sulfur rather than carbon substitution was observed with thiophenol. Fourth, in the reaction involving indole, the indole trimer **9** (8) was obtained along with the desired product **1g**. Indole is known to undergo trimerization in the presence of both mineral and Lewis acids (8, 9).



9

Characteristic spectral properties were noted for the newly prepared functionalized amino acid derivatives **1** in agreement with the proposed structural assignments (10, 11). In particular, the chemical shift value for the α-carbon proton ranged from δ5.34 to 5.90 in the ^{1}H n.m.r. spectra, while the corresponding methine carbon signal appeared between 49.20 and 57.65 ppm. Evidence for the proposed site of aromatic substitution was secured from both the ^{1}H and ^{13}C n.m.r. spectra. In each case, the proton chemical shift values as well as the proton-proton coupling patterns were in excellent agreement with previously reported compounds of comparable substitution patterns (8, 12). Moreover, in the ^{13}C n.m.r. spectra, the chemical shift values observed for the substituted aromatic carbon atoms were always downfield (6.0–20.0 ppm) versus the corresponding signal in the unsubstituted heterocycle (11). In several cases (compounds **1i** and **1k**) the ^{13}C n.m.r. assignments were aided by per-

forming the corresponding APT n.m.r. experiment (13).

CONCLUSIONS

A facile procedure has been developed for the synthesis of non-natural amino acid derivatives containing an electron-rich aromatic or heteroaromatic α-substituent using an amidoalkylation transformation. The reaction proceeded with high regioselectivity and permitted the use of the acid sensitive heterocycles: pyrrole, benzofuran, and indole. Significantly, this approach should be applicable for the preparation of peptides in which the peptide bond is formed prior to the introduction of the aromatic or heteroaromatic substrate. Optimization of the general reaction conditions (i.e., Lewis acid, solvent) should allow the synthesis of other α-substituted functionalized amino acid derivatives.

EXPERIMENTAL PROCEDURES

General methods

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra (i.r.) were run on either a Perkin-Elmer 1330 or a Perkin-Elmer 283 spectrophotometer and calibrated against the 1601 cm^{-1} band of polystyrene. Absorption values are expressed in wavenumbers (cm^{-1}). Proton (^1H n.m.r., 300 MHz) and carbon (^{13}C n.m.r,, 75 MHz) nuclear magnetic resonance spectra were taken on either a Nicolet NT-300 or a General Electric QE300 instrument. Chemical shifts are in parts per million (δ values) relative to tetramethylsilane (TMS) and coupling constants (*J* values) are in Hertz. Mass spectra were performed at the Eli Lilly Corporation, Indianapolis, Indiana, or by Dr. John Chinn at the Department of Chemistry, University of Texas at Austin. Elemental analyses were conducted at the Eli Lilly Corporation, Indianapolis, Indiana. Acetonitrile and triethylamine were distilled from CaH_2 and tetrahydrofuran and ethyl ether were distilled from Na/benzophenone. Furan, pyrrole, benzofuran, ethyl chloroformate, and isobutyl chloroformate were fractionally distilled prior to use. All other chemicals were of the highest grade available and were used without further purification. The mixed anhydride reactions as well as the amidoalkylation transformations using boron trifluoride etherate were run under anhydrous conditions. In these cases, all glassware was flame-dried under N_2, the solid starting materials were dried *in vacuo* prior to use, and the reactions were conducted under a positive pressure of N_2. Preparative flash column chromatography was run using Merck silica gel, grade 60, 230–240 mesh, 60 Å from Aldrich Chemical Company, Milwaukee, Wisconsin. Thin-layer chromatographic analyses were run on precoated silica G microscope slides (2.5 × 10 cm; Analtech No. 01521) or on precoated silica GHLF microscope slides (10 × 20 cm; Analtech No. 21521).

*Preparation of methyl 2-acetamido-2-methoxyacetate (**5a**)*

Sulfuric acid (95%, 4 mL, 70 mmol) was added to a methanolic solution (230 mL) of 2-acetamido-2-hydroxyacetic acid (**4**) (13.30 g, 100 mmol). The solution was stirred at room temperature (48 h), neutralized with solid $NaHCO_3$, filtered, and then the methanol was removed *in vacuo*. The pink oil was distilled under vacuum (70–120°, 0.6 torr) to give a colorless oil which was recrystallized from petroleum ether (35–60°) to yield 5.20 g (32%) of the desired product: R_f 0.52 (98:2 chloroform/methanol); m.p. 44–46°; i.r. (KBr) 3270, 2820, 1735, 1650 (br), 1505, 1205, 1110, 1090, 1010, 930, 900 cm^{-1}; ^1H n.m.r. ($CDCl_3$) δ2.08 (s, CH_3CO), 3.46 (s, OCH_3), 3.81 (s, $COOCH_3$), 5.54 (d, J = 9.3 Hz, CH), 6.70–6.80 (br d, NH); ^{13}C n.m.r. ($CDCl_3$) 22.98 (CH_3CO), 52.69 ($COOCH_3$), 56.48 (CH_3O), 78.16 (CH), 168.49 (CH_3CO), 170.67 ($COOCH_3$) ppm; mass spectrum, m/e (relative intensity) 162 (1), 146 (2), 131 (3), 118 (3), 102 (46), 88 (25), 60 (100).

Anal. calc. for $C_6H_{11}NO_4$: C 44.72, H 6.88, N 8.69. Found: C 44.46, H 7.14, N 8.72.

*Preparation of ethyl 2-acetamido-2-ethoxyacetate (**5b**)*

Sulfuric acid (95%, 8 mL, 140 mmol) was added to an ice cold ethanolic solution

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.