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Review Article

Proteinogenic Amino Acids Labelled with ^{15}N and/or ^{13}C for Application in Peptide Synthesis: A Short Review with a Comprehensive List of Published Derivatives

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Abstract: A review is given of the literature dealing with the most common protected derivatives of ^{15}N- and/or ^{13}C-labelled amino acids of interest in peptide synthesis. The list contains all such Boc-, Z- and Fmoc-amino acids as well as published methyl, ethyl, *t*-butyl and benzyl esters.

Keywords: Carbon-13; isotope-labelled amino acid; nitrogen-15; proteinogenic amino acid; stable isotope

INTRODUCTION

Amino acids labelled with the stable nuclei ^{15}N and/or ^{13}C have played important roles in various metabolic, biosynthetic and mechanistic studies during recent decades. Since the pioneering synthetic experiments were performed in this field [1–4], altogether over 150 isotopomers of the 20 species commonly found in proteins have been described. More recently, such amino acids have also been incorporated into proteins for use in structural studies by various NMR techniques [5]. Although the natural abundance of the ^{13}C nucleus is high enough (1.1%) to be useful in many cases without labelling, relatively large amounts of sample and long spectral accumulation times are required. The even lower natural abundance of ^{15}N (0.37%) further stresses the need for labelling in order to exploit this nucleus in such studies.

For the synthesis of isotopically labelled peptides for structural and other purposes, the corresponding protected amino acid derivatives are required. To date a considerable number of such have been described in the literature, most of which are not easy to retrieve without a systematic search. Therefore, these compounds are summarized for the first time in this review.

ORGANIZATION OF THE REVIEW

This review is restricted entirely to the proteinogenic amino acids. It comprises only the most frequently applied protected derivatives used in peptide synthesis. These include the Boc-, Z- and Fmoc-amino protected derivatives, the methyl, ethyl, *t*-butyl and benzyl esters and the *t*-butyl and benzyl ethers and thioethers. For these compounds the *Formula Index of Chemical Abstracts* has been systematically searched up to June 1994. A few other protected derivatives found during this search have been added. All of these compounds are collected in Table 1.

Table 1 is arranged alphabetically with respect to the involved amino acid derivatives. Both uniformly

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and specifically labelled isotopomers are listed and to identify them unambiguously their Chemical Abstracts Service (CAS)-registry numbers are also included, together with those of the labelled parent free amino acids. In most cases, some information about the preparation or origin of the compounds and other relevant comments is also given. Finally, a reference is given to the original literature.

The methods of preparation used reflect the development of both amino acid synthesis and protection during the last decades. Free amino acids prepared chemically as racemates and resolved by use of enzymes are coded as A. Others of unspecified or commercial origin are characterized by B or C. The use of microbiological or biosynthetic methods is denoted by D. Conversion of an amino acid or derivative thereof into another labelled one without resolution is coded E. Asymmetric or other direct synthesis is indicated by F or G, respectively. Non-routine derivatization is generally pointed out under comments.

COMMENTS

As shown in Table 1, nearly 100 isotopomers of derivatives of proteinogenic amino acids with relevance in peptide synthesis have been described in the literature to date. From them a considerable number of labelled peptides have been prepared and studied.

Further inspection of Table 1 demonstrates that no labelled derivatives with application in peptide synthesis have so far been prepared from the eight amino acids cystine, glutamic acid, histidine, isoleucine, lysine, methionine, threonine and tryptophan. For the remaining 11, excluding glycine, for which a large number of relevant species are available, an average of 5 different labelled protected derivatives that are of interest in this context have been made. Selectively ^{15}N-labelled derivatives exist for alanine, glycine, leucine, phenylalanine, tyrosine and valine only. Such can nowadays generally be made from commercial D-amino acids via the corresponding hydroxy acids [6,39]. For a trifunc-

Table 1 List of Amino Acid Derivatives, Labelled with ^{15}N and/or ^{13}C

No.	Derivative/pattern of labelling	CAS registry no./do. free amino acid	Preparation method	Comments	Ref.(s)
1	Boc-Ala	139952-87-7	E	Simultaneous protection	6
	^{15}N	25713-23-9			
2	Boc-Ala		F		7
	$^{15}N,1,2\text{-}^{13}C_2$	– – –			
3	Boc-Ala		F		7
	$^{15}N,1,2\text{-}^{13}C_2$, $3,3,3\text{-}D_3$	– – –			
4	Boc-Ala	72634-72-1	C	90% enriched	8
	U-^{13}C	100108-77-8			
5	Z-Ala	155256-73-8	B		9
	3-^{13}C	65163-25-9			
6	Fmoc-Ala	117398-49-9	C		10
	^{15}N	25713-23-9			
7	Ala-OME·HCl	75531-21-4	B	*ca.* 1%^{15}N	11
	^{15}N	25713-23-9			
8	Ala-OMe·HCl	137295-73-9	B		12
	1-^{13}C	21764-56-7			
9	Ala-OBzl·Tos	139929-02-5	E		6
	^{15}N	25713-23-9			
10	Z-Arg	141074-93-3	C		13
	1-^{13}C	81201-96-9			
11	Z-Arg(Pmc)	141074-94-4	C		13
	1-^{13}C	81201-96-9			
12	Fmoc-Arg(Pmc)	141074-96-6	C		13
	1-^{13}C	81201-96-9			
13	Arg(Pmc)	141074-95-5	C		13
	1-^{13}C	81201-96-9			

(continued)

Table 1 continued

No.	Derivative/pattern of labelling	CAS registry no./do. free amino acid	Preparation method	Comments	Ref.(s)
14	Boc-Asn	150464-76-9	E		14
	4-^{15}N	89695-60-3			
15	Boc-Asn-OBzl	150464-74-7	E		14
	4-^{15}N	89695-60-3			
16	Boc-Asp(OBzl)	72015-63-5	D	85% enriched	15
	U-^{13}C	55443-54-4			
17	Boc-Asp-(OBzl)-OSU	72015-64-6	D	85% enriched	15
	U-^{13}C	55443-54-4			
18	Z-Asp	86418-73-7	D	85% enriched	16
	U-^{13}C	55443-54-4			
19	Fmoc-Asp(O^{t}Bu)	141074-92-2	C		13
	3-^{13}C	68261-19-8			
20	Asp(O^{t}Bu)	– – –	C		13
	3-^{13}C	68261-19-8			
21	Asp(OBzl)	72015-62-4	D	85% enriched	15
	U-^{13}C	55443-54-4			
22	Z-Cys(S-Bzl)	79761-02-7	A		17
	3-^{13}C	138307-42-3			
23	Cys(S-Bzl)	136743-61-8	A		18
	1-^{13}C	– – –			
24	Cys(S-Bzl)	79761-09-4	A		17
	3-^{13}C	138307-42-3			
25	Boc-Gln	150464-77-0	E		14
	5-^{15}N	59681-32-2			
26	Boc-Gln-OBzl	150464-75-8	E		14
	5-^{15}N	59681-32-2			
27	Boc-Gly	106665-75-2	C	90% enriched	19
	^{15}N	7299-33-4			
28	Boc-Gly	145143-00-6	G	Simultaneous protection	20,21
	^{15}N,1-$^{13}C_2$	– – –			
29	Boc-Gly	145142-98-9	G	Simultaneous protection	20,21
	^{15}N,1,2-$^{13}C_2$	– – –			
30	Boc-Gly	145143-01-7	G	Simultaneous protection	20,21
	^{15}N,2-^{13}C	– – –			
31	Boc-Gly	97352-64-2	C	90% enriched	22
	1-^{13}C	20110-59-2			
32	Boc-Gly	65096-45-9	D	85% enriched	23
	U-^{13}C	67836-01-5			
33	Boc-Gly	145142-99-0	G	Simultaneous protection	20,21
	1,2-$^{13}C_2$	67836-01-5			
34	Boc-Gly	145143-02-8	G	Simultaneous protection	20,21
	2-^{13}C	20220-62-6			
35	Boc-Gly-$^{15}NH_2$	145143-03-9	G		20
	^{15}N,1,2-$^{13}C_2$	– – –			
36	Boc-Gly-$^{15}NH_2$	145143-04-0	G		20
	1,2-$^{13}C_2$	67836-01-5			
37	Boc-Gly-$^{15}NH_2$	145143-05-1	G		20
	2-^{13}C	20220-62-6			
38	Boc-Gly-ONp		G		24
	^{15}N	7299-33-4			

(*continued*)

Table 1 continued

No.	Derivative/pattern of labelling	CAS registry no./do. free amino acid	Preparation method	Comments	Ref.(s)
39	Z-Gly	67739-37-1	C	2.25% enriched	25
	1-^{13}C	20110-59-2			
40	Z-Gly	80058-03-3	C		26
	1,2-$^{13}C_2$	67836-01-5			
41	Fmoc-Gly	125700-33-6	C		27
	^{15}N	7299-33-4			
42	Ac-Gly	1449-79-2	B		28
	^{15}N	7299-33-4			
43	Ac-Gly	– – –	C		29
	1-^{13}C	20110-59-2			
44	Ac-Gly	64225-28-1	C		30
	2-^{13}C	20220-62-6			
45	Pht-Gly		G		31
	1-^{13}C	20110-59-2			
46	Trt-Gly-OMe	98292-02-5	C		32
	^{15}N	7299-33-4			
47	Gly-OMe	104576-76-3	C		33
	^{15}N	7299-33-4			
48	Gly-OMe·HCl	72634-71-0	C	60% enriched	8
	^{15}N	7299-33-4			
49	Gly-OMe	137248-37-4	B		12
	1-^{13}C	20110-59-2			
50	Gly-OMe·HCl	126614-97-9	C		34
	1,2-$^{13}C_2$	67836-01-5			
51	Gly-OEt	117500-42-2	C		35
	^{15}N	7299-33-4			
52	Gly-OEt·HCl	58420-99-8	?		36
	^{15}N	7299-33-4			
53	Gly-OEt	143203-16-1	G		37
	1-^{13}C	20110-59-2			
54	Gly-OEt·HCl	67739-40-6	C	2.25% enriched	25
	1-^{13}C	20110-59-2			
55	Gly-OEt.HCl	58420-91-0	?		36
	2-^{13}C	20220-62-6			
56	Gly-OBzl·Tos	114342-15-3	?		38
	2-^{13}C	20220-62-6			
57	BMTM-Gly-OEt[a]		G		7
	^{15}N,1,2-$^{13}C_2$	– – –			
58	Boc-Leu	146953-81-3	E	Simultaneous protection	39
	^{15}N	59935-31-8			
59	Boc-Leu	102636-64-6	C		40
	1-^{13}C	74292-94-7			
60	Boc-Leu		F		7
	^{15}N,1,2-$^{13}C_2$	– – –			
61	Z-Leu	125069-90-1	C	90% enriched	41
	1-^{13}C	74292-94-7			
62	Leu-OMe	– – –	C	90% enriched	19
	^{15}N	59935-31-8			
63	Leu-OEt	93523-68-3	A	90% enriched; (*R*)?	42
	1-^{13}C	74292-94-7			
64	Leu-OBzl	115721-58-9	C		40
	1-^{13}C	74292-94-7			

(continued)

Table I continued

No.	Derivative/pattern of labelling	CAS registry no./do. free amino acid	Preparation method	Comments	Ref.(s)
65	Boc-Phe	87713-13-1	C		43
	^{15}N	29700-34-3			
66	Boc-Phe		F		7
	$^{15}N,1,2\text{-}^{13}C_2$	– – –			
67	Boc-Phe		F		7
	$^{15}N,1,2\text{-}^{13}C_2,3,3\text{-}D_2$	– – –			
68	Boc-Phe-ONp		G		24
	^{15}N	29700-34-3			
69	Fmoc-Phe	125700-32-5	C		27
	^{15}N	29700-34-3			
70	Phe-OMe	– – –	C	90% enriched	19
	^{15}N	29700-34-3			
71	Phe-OEt	94601-37-3	C		29
	^{15}N	29700-34-3			
72	Z-Pro	58274-21-8	D	85% enriched	44
	$U\text{-}^{13}C$	– – –			
73	Z-Pro-NH_2	58274-22-9	D	85% enriched	44
	$U\text{-}^{13}C$	– – –			
74	Pro-NH_2	58274-20-7	D	85% enriched	44
	$U\text{-}^{13}C$	– – –			
75	Pro-OMe	97352-63-1	C		22
	carboxy-^{13}C	81202-06-4			
76	Boc-Ser	139623-02-2	?		45
	$3\text{-}^{13}C$	89232-77-9			
77	Fmoc-Ser	141074-87-5	C		13
	$1\text{-}^{13}C$	81201-84-5			
78	Boc-Tyr	87713-11-9	C		43
	^{15}N	35424-81-8			
79	Boc-Tyr	68882-36-0	A	90% enriched	46
	$2\text{-}^{13}C$	125718-74-8			
80	Boc-Tyr(Bzl)		F		7
	$^{15}N,1,2\text{-}^{13}C_2$	– – –			
81	Fmoc-Tyr	125700-34-7	C		27
	^{15}N	35424-81-8			
82	Tyr-OBzl·Tos	100790-33-8	D		47
	$3,5\text{-}^{13}C_2$	70479-98-0			
83	Tyr-OBzl·Tos	100790-35-0	D		47
	$4\text{-}^{13}C$	81201-90-3			
84	Boc-Val	141509-91-3	C		48
	^{15}N	59935-29-4			
85	Z-Val	52617-00-2	C		49
	^{15}N	59935-29-4			
86	Fmoc-Val	125700-35-8	C		27
	^{15}N	59935-29-4			

[a] BMTM stands for bis(methylthio)methylene.

tional amino acid it will probably be most economical to start with a derivative with a protecting group in the sidechain. The introduction of a ^{13}C nucleus in positions 1 and/or 2 of an amino acid is more demanding. Most derivatives of this type listed in the table have been made by standard methods from commercial amino acids. In this context it should be pointed out that relatively few authors have commented on the sterical purity of their starting materials and products. A considerable number of ^{13}C-labelled amino acids have also been made via their racemates and resolved using enzymatic meth-

ods. Even in such cases, however, the sterical purity should be checked by a sensitive, direct, preferentially chromatographic method. Otherwise, the ultimate sterical integrity of the labelled peptide product is endangered. Last but not least the challenging alternative of producing amino acids by asymmetric synthesis [50] should be mentioned. This approach has so far found limited application, but looks extremely promising [7]. It might turn out to be the method of choice for backbone-labelled amino acids, since all of the glycine isotopomers required as starting materials have already been quite easily made [20, 21].

In conclusion, it appears that the road to specifically labelled amino acids and from them to the corresponding peptides is now wide open. An extra bonus of the selective labelling approach resides in the fact that, in principle, it can be combined with the introduction of additional nuclei.

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