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OPTIMIZATION OF THE PAPAIN CATALYZED ESTERIFICATION OF AMINO ACIDS BY ALCOHOLS AND DIOLS

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Abstract: Esterification of Boc-Alanine and Boc-Aspartic acids by alcohols $C_nH_{2n+1}OH$ and diols $HO(CH_2)_nOH$ with immobilized papain (XAD-7 or Sepharose) is discussed. Great improvement is obtained for the esterification of Boc-Ala-OH if papain is entrapped in XAD-7. For example no esterification is observed with 1-decanol if free papain is used whereas a 55% yield is obtained with papain immobilized on XAD-7. Esterification of Boc-Asp-OH with diols has been achieved with papain immobilized on Sepharose. In the case of ethyleneglycol no condensation could be observed with free papain or papain on XAD-7 whereas a 40% yield of esterification was obtained with papain on Sepharose.

The recent realization that enzymes can function not only in aqueous medium but also in organic solvents has been of great interest[1]. For example, lipases have been used in anhydrous organic solvents for a wide range of stereoselective transformations[2,3]. However, although the esterification of acids by lipases has been known for a long time, attempts to esterify amino acids have failed[4].

Most proteases catalyze not only their natural hydrolysis reaction of peptide bonds but also catalyze peptide bond formation[5]. Ester synthesis by proteases can also be achieved. For example the ethyl esters of N-acetylphenylalanine, tryptophan and tyrosine have been prepared with α chymotrypsin as a catalyst under biphasic conditions[6-8]. Such amino acid esters are very useful in kinetically controlled enzymatic peptide synthesis by thiol or serine proteases[9]. Furthermore long chain alkyl esters of amino acids are of medicinal value and methods to prepare them are still being developped[10].

In a previous report[11] we showed that papain itself offers a great advantage over α chymotrypsin in esterification reactions since a wide range of Boc-protected amino acids could be esterified in good yields (biphasic system, pH 4.2, 37°C). Recently it was confirmed that

papain is a good catalyst for the esterification of Z-Ala-OH[12].

No side chain protection was needed in the papain catalyzed esterification of aspartic and glutamic acids which is of great advantage since the chemical synthesis of the α amino ester usually involves several steps (however a simple method for preparing the α amino esters of aspartic and glutamic acids by activation with alkyl chloroformate was recently described[13]).

With Boc-Ala-OH as the substrate and papain as the catalyst we also showed that monoesters could be obtained with the diols $HO(CH_2)_nOH$[14]. Esterification works well up to n = 10. With the monoalcohols $C_nH_{2n+1}OH$ esterification was observed only for n = 1 to 6.

In this paper we show that papain immobilized on XAD-7 [a neutral cross-linked poly(methylacrylate)] greatly improves the yields of esterification for Boc-Ala-OH. The esterification of Boc-Asp-OH with these long chain alcohols is also described. In this case the use of Sepharose for immobilization of the enzyme is discussed.

With papain immobilized on XAD-7 the water content is minimized. The immobilization is performed as described with XAD-8 as support[15]. Competition between water and alcohol should decrease and yields of esterification increase. In fact if Boc-Ala-OH is used as the substrate and methylene chloride as the solvent the yields of esterification by the alcohols $C_nH_{2n+1}OH$ greatly increase. Condensation takes place up to n = 16 (51% yield: Table I). This is to be compared with esterification using the free enzyme where no condensation took place for n = 10 and 12.

Table I. Esterification of Boc-Ala-OH by papain immobilized on XAD-7

ROH	Conditions[a.b]	Boc-Ala-OR[c] papain on XAD-7 Ester	yield %	Boc-Ala-OR[d] free papain (yield %)	m.p. °C	$[\alpha]^e_D$
$C_8H_{17}OH$	2/0.2	1	85	21		-33
$C_{10}H_{21}OH$	2/0.4	2	55	0		-30
$C_{12}H_{25}OH$	2/0.4	3	55	0		-28
$C_{16}H_{33}OH$	2/0.4	4	51	0	40	-24
$C_{18}H_{37}OH$	2/0.4	5	26	0	43	-23
Solketal	2/0.2	6	70	70	93	-19
$HO[CH_2]_2OH$	20/0.2	7	78	74	86	-35
$HO[CH_2]_{10}OH$	2/0.5	8	68	66		-28
$HO[CH_2]_{12}OH$	2/0.5	9	63	26	33	-21
$HO[CH_2]_{16}OH$	3/0.5	10	23	0	52	-22
$CH_3CHOHCH_2OH$	10/0.2	11	75	75		

a) The esterifications were performed on 0.5 mmole of Boc-Ala-OH and 100 mg of papain immobilized on XAD-7, 37°C, 18 h. b) Amounts of CH_2Cl_2/ROH in ml or g if the alcohol is solid. c) After purification. d) ref. 14. e) c, 1 in methanol; 23°C

Improvement of the condensation is also observed for the diols $HO[CH_2]_nOH$ since a 63% yield of monoesterification is obtained for n = 12 as compared to 26% with the free enzyme.

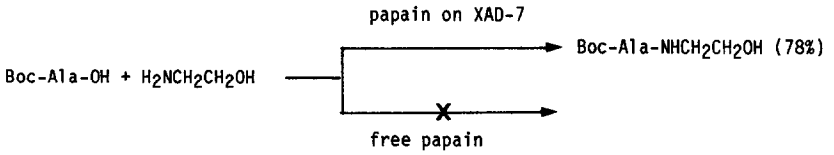
Note that the amount of alcohol used in the esterification with immobilized papain is greatly reduced since only a 5-fold excess is necessary as compared to the 25-fold excess in the free enzyme catalyzed synthesis. This facilitates the purification process and can be of interest for more expensive alcohols. It could also be useful for the preparation of labelled amino acids esters.

Both enantiomers of 1,2-propanediol (R and S) give good yields of esterification and no enantioselectivity is observed when the racemic mixture is used. Furthermore a complex mixture of esters is obtained. The R enantiomer itself gives 40% of ester B whereas the S enantiomer gives only 8% of B.

$$\text{Boc-Ala-OH} + \text{HOCH}_2\text{CH(CH}_3\text{)OH} \longrightarrow \underset{\text{A}}{\text{Boc-Ala-O-CH}_2\text{CH(CH}_3\text{)OH}} + \underset{\text{B}}{\text{Boc-Ala-O-CH(CH}_3\text{)CH}_2\text{OH}}$$

The unexpected isomer B is probably due to a transposition of the "normal" ester A since secondary alcohols condense very poorly with papain as a catalyst. The same mixture is observed in the deprotection of the benzyl derivative of ester B (prepared by a chemical method).

With ethanolamine itself and 2-aminopropanol the amino acid amide is formed. Good yields of condensation are observed if papain is entrapped in XAD-7 whereas with the free enzyme no condensation takes place if the amino alcohol is unprotected[14]



However, with the racemic 2-aminopropanol a diastereoisomeric mixture is produced and no enantioselectivity is observed. The same kind of amide bond has been obtained by the carboxypeptidase Y catalysed condensation of Bz-Ala-OMe with ethanolamine at pH 9.5[16].

Isopropylidene glycerol (solketal) gives a good yield of esterification but here again no enantioselectivity is observed. Both R and S enantiomers condense with Boc-Ala-OH to give a 70% yield. In the condensation with 2-benzyl glycerol a diastereoisomeric mixture of esters in a ratio of 1/1 is obtained, clearly showing no enantioselectivity.

The case of aspartic acid is quite interesting since only the α carboxyl group is esterified. With the diols $HO(CH_2)_nOH$ only the monoester is formed (< 3% of the diester), as with alanine

$$\text{Boc-Asp-OH} + \text{HO(CH}_2\text{)}_n\text{OH} \longrightarrow \underset{< 3\%}{\text{Boc-Asp-O(CH}_2\text{)}_n\text{OH}} + \text{Boc-Asp-O(CH}_2\text{)}_n\text{O-Asp-Boc}$$

With aspartic acid Sepharose is a good support for the immobilization of papain. For example esterification of Boc-Asp-OH with ethyleneglycol occurs if papain is supported on Sepharose whereas no condensation takes place with papain immobilized on XAD-7 or with the free enzyme. Yield improvement is also observed for 1,4-butanediol. In fact it seems that the esterification is nearly total for these two diols as seen by thin layer chromatography but difficulties are encountered in the purification process. With longer-chain diols esterification occurs if papain is immobilized on XAD-7 but better yields are observed with Sepharose as support, up to n = 12 (Table II).

With the monoalcohols $C_nH_{2n+1}OH$ poor yields of esterification are obtained when n is greater than 6 (41% for n = 8, 21% for n = 10), either with papain immobilized on XAD-7

or with Sepharose as support (Table II).

As expected enzyme-catalyzed esterification of N-protected DL amino acids was found to apply only to the L enantiomer.

In summary, the papain catalyzed esterification of amino acids by the diols $HO(CH_2)_nOH$ works well in the case of Boc-Ala-OH and Boc-Asp-OH. XAD-7 is a good support for papain in the esterification of Boc-Ala-OH, whereas Sepharose is a better support for the esterification of Boc-Asp-OH. Furthermore, if papain is immobilized on Sepharose no esterification occurs for alanine. In fact it can be expected that esterification is favoured when papain is amply supplied by the substrate molecules i.e. when the substrate concentration in the microenvironment is high. With the rather hydrophobic support XAD-7 a rather hydrophobic substrate (alanine) is preferred, whereas with the hydrophilic Sepharose support a hydrophylic substrate (aspartic acid) is better. Results obtained with biocatalyst entrapping gels of desired hydrophobicity and hydrophilicity show the same tendancy[18].

For the two amino acids studied, esterification by the alcohols $C_nH_{2n+1}OH$ is more difficult than by the long chain diols $HO(CH_2)_nOH$, although for alanine the use of papain immobilized on XAD-7 greatly improved the yield of esterification. The esterification of aspartic acid with monoalcohols worked well only for n = 1 to 6.

Table II. Esterification of Boc-Asp-OH by papain

ROH	Conditions[a.b]	Boc-Asp-OR[c] papain on XAD-7 Ester	yield %	Boc-Asp-OR[c.d] papain on Sepharose yield %	Conditions[e]	m.p. °C	$[\alpha]_D^f$
CH_3OH	5/0.3	14	70	71	0.3/5/0.5	89	-19
C_2H_5OH	5/0.3	15	70	73	0.3/5/0.5[g]	107	-22
C_4H_9OH	5/0.3	16	73	74	0.3/5/0.5	74	-24
$C_6H_{13}OH$	1/0.3	17	73	70	0.3/2/0.5	66	-22
$C_8H_{17}OH$	1/0.3	18	41	31	0.3/2/0.5	70	-18
$C_{10}H_{21}OH$	1/0.3	19	10	21	0.3/2/0.5		-13
Solketal	1/0.3	20	22	38	0.3/2/0.5	86	-17
$HO[CH_2]_2OH$	50/0.2	21	0	39	0.3/50/0.5	126	-14
$HO[CH_2]_4OH$	10/0.5	22	21	62	0.3/20/0.2	61	-22
$HO[CH_2]_6OH$	2/0.5	23	58	80	0.3/10/0.12		-20
$HO[CH_2]_8OH$	2/0.5	24	62	78	0.3/5/0.3		-17
$HO[CH_2]_{10}OH$	2/0.5	25	62	72	0.3/5/0.3		-16
$HO[CH_2]_{12}OH$	2/0.5	26	47	72	0.3/5/0.3	53	-16

a) The esterifications were performed on 0.5 mmole of Boc-Asp-OH and 100 mg of papain immobilized on XAD-7[15], 37°C, 18 h. b) Amounts of CH_2Cl_2/ROH in ml or g if the alcohol is solid. c) After purification. d) ref. 17 for the immobilization on Sepharose. e) Amount of buffer/CH_2Cl_2/ROH. f) c, 1 in methanol; 23°C. g) ref. 11

EXPERIMENTAL

M.p.s. were obtained using a microscope hot-stage and are uncorrected. Optical rotation were measured at 589 nm (sodium line) on a Perkin-Elmer 241 MC polarimeter. Mass spectra were obtained from a Nermag R 10-10C apparatus (chemical ionisation with NH_3, 90 ev). ^1H n.m.r. spectra were recorded at 250 or 400 MHz on a Brucker instrument in $CDCl_3$ with Me_4Si as the internal standard. Advancement of the reactions and purity of the esters were tested by t.l.c. with precoated silica gel (Merck, silica gel $60F_{254}$ plates) and appropriate mixtures of methylene chloride-methanol or methylene chloride-methanol-acetic acid. The spots were developped by spraying with ninhydrin or with phosphomolybdic acid, followed by heating. The Boc-Ala-OR derivatives were purified by chromatography on LH20 (Pharmacia) using T.H.F. as the eluant, followed by chromatography on silica gel (Merck: 0.040-0.063 mm) using methylene chloride-methanol as the eluant. The Boc-Asp-OR derivatives were purified by chromatography on silica gel using methylene chloride-methanol-acetic acid as the eluant (96/4/0.1).

Papain was purchased from Sigma Chemical Co as a crude powder with a specific activity of 2.9 units per mg of solid. The powder was used without further purification. Boc-Amino acids were purchased from Novabiochem. XAD-7 and Sepharose 4B-200 were obtained from Sigma as well as (R)-isopropylidene glycerol and (S) 1,2-propanediol. (S)-isopropylidene glycerol was prepared from D-Mannitol as described[19]. (R) 1,2-propanediol was synthetized by Baker's yeast reduction of hydroxyacetone[20]. Cysteine was purchased from Aldrich Chem. Co. Methylene Chloride (Aldrich) was distilled over sodium carbonate.

Preparation of the enzymatic catalyst

Papain was entrapped in XAD-7 or Sepharose 4B as described below. a) Papain (100 mg) was stirred with 5 ml 1 M Mc Illvaine buffer (pH 4.2) and 400 mg of XAD-7 for 10 h[15]. The entrapped enzyme was filtered and transferred to a double-walled screw-cap reactor with 17 mg of cysteine and 30 µl of 1 M EDTA (ethylene diamine tetraacetic acid tetrasodium salt, trihydrate). The mixture was incubated 1 h at 37°C. b) Swollen Sepharose 4B (2 ml)[17] was washed with water and with 1 M Mc Illvaine buffer (pH 4.2). Then the resin was filtered, the resulting gel was cut into pieces and transferred to a reactor. To the Sepharose was added 0.3 ml of buffer containing 100 mg of papain, 17 mg of cysteine and 30 µl of 1 M EDTA. The immobilized enzyme was then incubated 30 mn at room temperature.

Enzymatic esterification

0.5 mmole of Boc-amino acid (alanine: 95 mg or aspartic acid: 116.5 mg) was added to the reactor containing the entrapped enzyme with methylene chloride and the alcohol, in the proportions given in Table I and II. The mixture was shaken with an orbit-shaker at 200 r.p.m. and 37°C for 18 h. After completion of the reaction, the enzymatic catalyst was filtered. The organic phase was dried, evaporated and the esters were purified as described above. In the esterification of Boc-Ala-OH only XAD-7 was used as support for papain whereas with Boc-Asp-OH either XAD-7 or Sepharose 4B were used.

Boc-Alanine octyl ester 1

M + H^+ , 302. (Found: C, 63.44; H, 10.31; N, 4.66. $C_{16}H_{31}NO_4$ requires C, 63.75; H, 10.36; N, 4.64 %). δ_H (250 MHz) 0.92 (3H, t, J 7 Hz), 1.28 (10H), 1.41 (3H, d, J 7 Hz), 1.47 (9H, s,), 1.68 (2H, m), 4.18 (2H, m), 4.32 (1H, m), 5.35 (1H, NH).

Boc-Alanine decyl ester 2

M + H^+, 330. (Found: C, 65.69; H, 10.76; N, 4.20. $C_{18}H_{35}NO_4$ requires C, 65.62; H, 10.71; N, 4.25 %). δ_H (250 MHz) 0.92 (3H, t, J 7 Hz), 1.28 (14H), 1.43 (3H, d, J 7 Hz), 1.46 (9H, s), 1.68 (2H, m), 4.22 (2H, m), 4.32 (1H, m), 5.28 (1H, NH).

Boc-Alanine dodecyl ester 3

M + H^+, 358. (Found: C, 67.07; H, 11.05; N, 3.94. $C_{20}H_{39}NO_4$ requires C, 67.18; H, 10.99; N, 3.91%). δ_H (400 MHz) 0.92 (3 H, t, J 7 Hz), 1.28 (18 H), 1.41 (3H, d, J 7 Hz), 1.46 (9H, s), 1.68 (2H, m), 4.15 (2H, m), 4.32 (1H, m), 5.35 (1H, NH).

Boc-Alanine cetyl ester 4

M + H^+, 414. (Found: C, 70.03; H, 11.51; N, 3.36. $C_{24}H_{47}NO_4$ requires C, 69.68; H, 11.45; N, 3.38%). δ_H (400 MHz) 0.92 (3H, t, J 7 Hz), 1.28 (26H), 1.41 (3H, d, J 7 Hz), 1.47 (9 H, s), 1.68 (2H), 4.17 (2H, m), 4.28 (1H, m), 5.30 (1H, NH).

Boc-Alanine octadecyl ester 5

M + H^+, 442. (Found: C, 70.56; H, 11.72; N, 3.20. $C_{26}H_{51}NO_4$ requires C, 70.70; H, 11.63; N, 3.17%). The n.m.r. spectrum is similar to the one of ester 4.

Boc-Alanine isopropylidene glyceryl ester 6

A 70% yield of isopropylidene glycerylester was obtained from racemic isopropylidene glycerol and from the R or S enantiomers. M + H^+, 304. (Found: C, 55.66; H, 8.25; N, 4.58. $C_{14}H_{25}NO_6$ requires C, 55.43; H, 8.30; N, 4.61%); δ_H (400 MHz) 1.37-1.45 (3H), 1.43 (3H, d, J 7 Hz), 1.47 (9H, s), 3.82-4.1 (2H, m), 4.2 (2H, m), 4.33 (2H, m). Note that the esters from R (or S) isopropylidene glycerol display the same n.m.r. spectrum.

Boc-Alanine 1-hydroxyethyl ester 7

M + H^+, 234. (Found: C, 51.76; H, 8.16; N, 6.04. $C_{10}H_{19}NO_5$ requires C, 51.49; H, 8.21; N, 6.00%). δ_H (250 MHz) 1.39 (3H, d, J 7 Hz), 1.43 (9H, s), 3.79 (2H, m), 4.26 (3H, m), 5.35 (1H, NH).

Boc-Alanine 1-hydroxydecyl ester 8

M + H^+ 346. (Found: C, 62.91; H, 10.16; H, 4.03. $C_{18}H_{35}NO_5$ requires C, 62.57; H, 10.21; N, 4.05%). δ_H (250 MHz) 1.22 (12H), 1.32 (3H, d, J 7 Hz), 1.41 (9H, s), 1.55 (4H, m), 3.57 (2H, t, J 7 Hz), 4.07 (2H, dd, J_1 7 Hz, J_2 2 Hz), 4.25 (1H, m), 5.32 (1H, NH).

Boc-Alanine 1-hydroxydodecyl ester 9

M + H^+, 374. (Found: C, 64.00; H, 10.58; N, 3.72. $C_{20}H_{39}NO_4$ requires C, 64.31; H, 10.52; N, 3.75%). δ_H (400 MHz) 1.26 (16H), 1.33 (3H, d, J 7 Hz), 1.43 (9H, s), 1.58-1.68 (4H, m), 3.57 (2H, t, J 7 Hz), 3.99 (2H), 4.28 (1H, m), 5.38 (1H, NH).

Boc-Alanine 1-hydroxycetyl ester 10

M + H^+, 430. (Found: C, 66.76; H, 11.08; N, 3.24. $C_{24}H_{47}NO_5$ requires C, 67.09; H, 11.02; N, 3.26%). δ_H (400 MHz) 1.24 (24H), 1.32 (3H, d, J 7 Hz), 1.43 (9H, s), 3.57 (2H, t, J 7 Hz), 4.02 (2H), 4.19 (1H, m), 5.36 (1H, NH).

Boc-Alanine 2-hydroxypropyl ester 11

A 75% yield was obtained from the R ans S enantiomers or the racemic 1,2-propanediol. M + H^+, 248. The n.m.r. shows a mixture of primary and secondary amino acid esters A and B (see text). For the racemic alcohol we find the following chemical shifts for the esters: δ_H (400 MHz) 1.44 (d, J 7 Hz), 1.24 (d, J 7 Hz) (A: 65%), 1.27 (d, J 7 Hz). 1.42 (d, J 7 Hz) (B: 20%), 1.28 (d, J 7 Hz) (B: 15%), 1.49 (s), 3.64, 3.73 (B), 4.07 (m), 4.19 (m), 4.32 (m) (A + B). The same mixture of esters A + B is obtained by deprotection of Boc-Ala-$OCH(CH_3)CH_2OCH_2C_6H_5$ by Pd/C. This ester was synthetized from Boc-Ala-OH and $HOCH(CH_3)CH_2OCH_2C_6H_5$ by the DCC/DMAP method[21].

Boc-Alanine 1-hydroxyethyl amide: Boc-Ala-$NHCH_2CH_2OH$ 12

$[\alpha]_D$ = - 17 (c1, MeOH). M + H^+, 233. (Found: C, 51.35; H, 8.71; N, 12.15. $C_{10}H_{20}N_2O_4$ requires C, 51.70; H, 8.68; N, 12.06%). δ_H (250 MHz) 1.32 (2H, d, J 7 Hz), 1.40 (9H, s), 3.38 (2H, m), 3.65 (2H, t, J 5 Hz), 4.13 (1H, m), 5.38 (1H, NH). Acetylation of amide 12 shows unambiguously the structure of the product since only the chemical shift of the CH_2OH group is displaced from 3.65 to 4.2 ppm.

Boc-Alanine 2-hydroxymethylethyl amide: Boc-Ala-$NHCH(CH_3)CH_2OH$ 13

m.p. = 65°C; $[\alpha]_D$ = - 28 (c1, MeOH). M + H^+, 247. (Found: C, 53.39. H, 8.95; N, 11.31. $C_{11}H_{22}N_2O_4$ requires C, 53.64; H, 8.90; N, 11.37%). δ_H (250 MHz) 1.13 (3H, d, J 7 Hz), 1.33 (3H, d, J 7 Hz), 1.41 (9H, s), 3.48-3.62 (2H, m), 4.08 (2H, m), 5.16-5.28 (1H, NH). If D_2O is added to the solution the two diastereo-isomers are clearly seen on the two methyl groups at 1.13 and 1.33 ppm in the proportion 40/60. Here again acetylation is performed. The chemical shift of the CH_2OH group is displaced from 3.48-3.62 ppm to 4.05 ppm.

Boc-Aspartic α methyl ester 14

M + H^+, 248. (Found: C, 48.64; H, 7.05; N, 5.64. $C_{10}H_{17}O_6N$ requires C, 48.58; H, 6.93; N, 5.66%). δ_H (250 MHz) 1.45 (9 H, s), 2.94 (2 H, dd, J_{AB} 17 Hz), 3.78 (3 H, s), 4.53 (1 H, m), 5.5 (1 H, NH).

Boc-Aspartic α butyl ester 16

M + H^+, 290. (Found: C, 53.77; H, 8.03; N, 4.93. $C_{13}H_{23}NO_6$ requires C, 53.96; H, 8.01; N, 4.84%). δ_H (400 MHz) 0.87 (3H, t, J 7 Hz), 1.38 (2H, m), 1.43 (9H, s), 1.62 (2H), 2.92 (2H, dd, J_{AB} 17 Hz), 4.12 (2H, t, J 7 Hz), 4.53 (1H, m), 5.3 (1H, NH).

Boc-Aspartic α hexyl ester 17

M + H^+, 318. (Found: C, 56.49; H, 8.61; N, 4.45. $C_{15}H_{27}NO_6$ requires C, 56.76; H, 8.57; N, 4.41%). δ_H (250 MHz) 0.87 (3H, t, J 7 Hz), 1.24 (6H), 1.42 (9H, s), 1.58 (2H), 2.92 (2H, dd, J_{AB} 17 Hz), 4.12 (2H, t, J Hz), 4.53 (1H, m), 5.0 (1H, NH).

Boc-Aspartic α octyl ester 18

M + H^+, 346. (Found: C, 58.82; H, 9.07; N, 4.03. $C_{17}H_{31}NO_6$ requires C, 59.10; H, 9.04; N, 4.05%). δ_H (250 MHz) 0.87 (3H, t, J 7 Hz), 1.24 (10H), 1.42 (9H, s), 1.61 (2H), 2.92 (2H, dd, J_{AB} 17 Hz), 4.14 (2H, t, J 7 Hz), 4.57 (1H, m), 5.1 (1H, NH).

Boc-Aspartic α decyl ester 19

M + H^+, 330. (Found: C, 60.79; H, 9.49; N, 3.72. $C_{19}H_{35}NO_6$ requires C, 61.10; H, 9.44; N, 3.75%). δ_H (400 MHz) 0.87 (3H, t, J 7 Hz), 1.24 (24H), 1.42 (9H, s), 1.62 (2H), 2.92 (2H, dd, J_{AB} 17 Hz), 4.12 (2H, t, J 7 Hz), 4.57 (1H, m), 5.23 (1H, NH).

Boc-Aspartic α isopropylidine glyceryl ester 20

M + H^+, 349. (Found: C, 51.45; H, 7.56; N, 4.92. $C_{15}H_{26}NO_8$ requires C, 51.71; H, 7.52; N, 4.02%). δ_H (400 MHz) 1.39-1.46 (6H), 1.48 (9H, s), 2.92 (2H, dd, J_{AB} 17 Hz), 3.78 (m), 4.01 (m), 4.25 (m), 4.35 (m, 5H), 4.62 (1H, m), 5.7 (1H, NH).

Boc-Aspartic α 1-hydroxyethyl ester 21

Difficulties in the purification of this ester are sometimes encountered since it is not easy to get rid of ethylene glycol. M + H^+, 278. (Found: C, 47.31; H, 6.95; N, 5.02. $C_{11}H_{19}NO_7$ requires C, 47.65; H, 6.90, N, 5.05. δ_H (CD_3OD, 400 MHz) 1.53 (9H, s), 2.91 (2H), 3.83 (2H, t, J 5 Hz), 4.29 (2H, m), 4.60 (1H), 5.10 (1H, NH). An authentic sample of this ester was synthetized by reacting Boc-Asp(OBzl)OH with ethylene glycol followed by debenzylation of the ester formed.

Boc-Aspartic α 1-hydroxybutyl ester 22

Here again difficulties are encountered in the purification. M + H^+, 306. (Found; C, 51.53; H, 7.58; H, 4.58. $C_{13}H_{23}NO_7$ requires C, 51.14; H, 7.59; N, 4.58%). δ_H (250 MHz) 1.43 (9H, s), 1.62 (4H), 2.92 (2H, dd, J_{AB} 17 Hz), 3.64 (2H, t, J 5 Hz), 4.16-4.23 (2H), 4.55 (1H), 5.85 (1H, NH).

Boc-Aspartic α 1-hydroxyhexyl ester 23

M + H^+, 334. (Found: C, 53.77; H, 8.19; N, 4.15. $C_{15}H_{27}NO_7$ requires C, 54.04; H, 8.16; N, 4.20%). δ_H (400 MHz) 1.41 (4H), 1.47 (9H, s), 1.6 (2H, m), 1.72 (2H, m), 2.92 (2H, dd, J_{AB} 17 Hz), 3.7 (2H, t, J 5 Hz), 4.13-4.28 (2H, m), 4.6 (1H), 5.65 (1H, NH).

Boc-Aspartic α 1-hydroxyoctyl ester 24

M + H^+, 362. (Found: C, 56.19; H, 8.59; N, 3.84. $C_{17}H_{31}NO_7$ requires C, 56.49; H, 8.64; N, 3.87%). δ_H (250 MHz) 1.3 (8H), 1.43 (9H, s), 1.52-1.6 (4H), 2.92 (2H, dd, J_{AB} 17 Hz), 3.62 (2H), 4.08-4.22 (2H), 4.52 (1H, m), 5.6 (1H, NH).

Boc-Aspartic α 1-hydroxydecyl ester 25

M + H^+, 390. (Found: C, 58.92; H, 9.10; N, 3.55. $C_{19}H_{35}NO_7$ requires C, 58.59; H, 9.05; N, 3.59%). δ_H (250 MHz) 1.3 (12H), 1.43 (9H, s), 1.58 (4H), 2.92 (2H, dd, J_{AB} 17 Hz), 3.63 (2H, t, J 5 Hz), 4.13 (2H, m), 4.52 (1H, m), 5.58 (1H, NH).

Boc-Aspartic α 1-hydroxydodecyl ester 26

M + H^+, 418. (Found: C, 60.05; H, 9.45; N, 3.32. $C_{21}H_{39}NO_7$ requires C, 60.40; H, 9.41; N, 3.35%). δ_H (400 MHz) 1.3 (16H), 1.5 (9H, s), 1.59 (2H, m), 1.66 (2H, m), 2.90 (2H, dd, J_{AB} 17 Hz), 3.68 (2H, t, J 5 Hz), 4.18 (2H, m), 4.6 (1H, m), 5.58 (1H, NH).

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