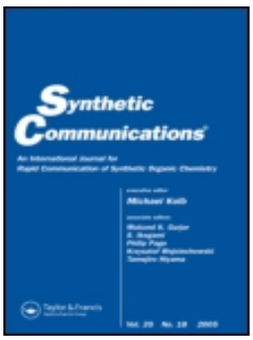

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A SIMPLE, GENERAL AND EFFICIENT METHOD FOR O AND N-RETINOYLATION. APPLICATION TO THE SYNTHESIS OF 2-RETINOYL-LECITHIN.

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ABSTRACT : The synthesis of the new 1-stearoyl-2-retinoyl-glycero-3-phosphorylcholine by coupling of retinoic acid and lysolecithin with DCC-DMAP (1.2 eq.) is reported. This method is applied to O and N-retinoylation of uncharged organic substrates such as aliphatic alcohols, free hydroxyl anomeric sugars, aromatic amines and C-protected α-aminoacids.

All-*trans* retinoic acid (ATRA) is a metabolite of vitamin A alcohol (retinol) and functions through its interactions with nuclear proteins retinoic acid receptors (RAR). Amongst its numerous biological activities, ATRA is involved in normal organogenesis, it modulates the growth and differentiation of various normal and malignant cells[1] by changes in gene expression.[2]

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The immune enhancer activity of ATRA has been shown to potentiate antibody responses to T-cell-dependent antigens, to increase lymphocyte proliferation responses to antigens and mitogens and to inhibit apoptosis.[3] But vitamin A and its analogs have also an endothelium-protective activity and an antiperoxidative effect[4] and they contribute to maintenance of the differentiated state in epithelial tissues.[5]

The usefulness of vitamin A and related retinoids in therapy of some infectious diseases is exemplified by the properties of some retinamides or retinoyl aminoacids (derived from leucine, alanine and phenylalanine) which reduce the carcinogen-induced cancers *in vivo* (bladder, mammary gland and pancreas) or which are cytotoxic for human epidermoid carcinoma cells in culture.[6]

But if retinoic acid and its synthetic analogs are now to be considered as potential drugs for treatment of oncologic and dermatologic diseases,[7] they have also been associated with serious side effects and have limited duration, at least in one form of leukemia (acute promyelocytic leukemia, APL) due to reduced serum concentration after prolonged treatment. The biological problems associated with the use of such substances can be solved by combining bioavailability in a wide range of aqueous cell compartments with a reduction of their toxic and teratogenic effects.[8] In this way, sugar and uronate derivatives of retinoic acid were prepared but only in minute amounts and some of them showed an activity similar to retinoic acid.[8] On the other side, liposomal as a possible formulation of ATRA administered intravenously, provide potential pharmaceutical advantages over the oral formulation in the treatment of malignant cells.[1]

Then, it is obvious that esterification or amidification of ATRA are two ways of potential useful derivatization of this compound. Thus, depending on the nature and functionnalities of the derivatizing agent it will be possible to modulate the biodisponibility and to target the activity of retinoids. As far as we are concerned with the various biological properties of retinoic acid and its derivatives,[9] we describe herein a significant improvement of some current methods used in retinoylation procedure and for the first time we report the synthesis of 1-stearoyl-2-retinoyl-glycero-3-phosphorylcholine **3**.

Amongst the various methods available for activation of the carboxylic moiety of ATRA **1**, retinoyl chloride or fluoride suffer from severe limitations in the purification of these most reactive intermediates.[10-16] On the other hand, the Mitsunobu reaction or the BOP activation used in the retinoylation of very specific substrates such as nucleosides give modest yields.[9,17] Retinoylimidazole is a well-defined and cristalline derivative of ATRA which give in some cases good yields in esterified compounds.[18-21] At last, the carbodiimide-DMAP or HOBT catalysed method has been reported to give erratic yields in the case of retinoylation.[17,22,23]

In order to achieve the synthesis of lecithins modified in the fatty acid residue by a retinoyl moiety at 2-position of the glycerol backbone, we elected at first to use two retinoylating agents namely chloride and imidazolide respectively.

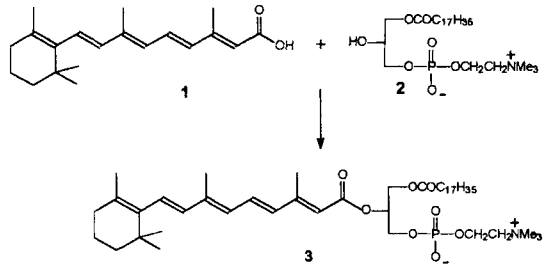
The starting lysolecithin **2** being the substrate to be retinoylated was synthesized by currently available known procedures[24] from the egg-yolk lipid content. Fractionation using a silica gel column chromatography gave a mixture of

lecithins which was fully deacetylated by tetrabutylammonium hydroxide to glycerophosphorylcholine which in turn was stearoylated to the pure monolecithin.[25] This later compound was subjected to the specific deacylation by use of phospholipase A_2 (PLA_2 from *Crotaleus Adamanteus*) and gave the lysolecithin **2**.

In our hands, the retinoyl chloride[11] (synthesized from retinoic acid and PCl_3 in benzene and Et_3N) or the cristalline imidazolide[20] both gave unsuccessful results when reacted with compound **2**.

Then we turned out towards the direct coupling between ATRA **1** and lysolecithin **2** by use of the well-documented DCC method.[26] This reaction is known to be catalysed by 4-DMAP and a typical experiment was performed under these usual conditions but gave disappointingly the N-retinoylurea **11** as the sole product.

SCHEME 1

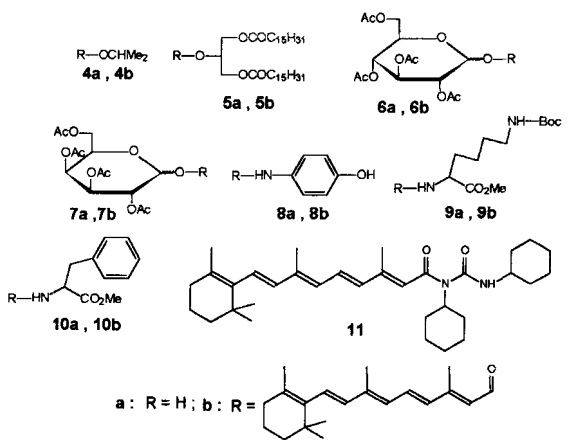


At this stage, we tried a similar approach but in going from the previous catalytic amount of 4-DMAP to a 1.2 molecular equivalent of this strong non-nucleophilic base and a 12 hrs reflux in chloroform (scheme 1).

This change in reaction conditions afforded successfully the required compound **3** in a 50% overall yield after purification and suppressed the unwanted formation of N-retinoylurea **11** even as a by-product.

The method which we applied to an amphiphilic molecule such as the lysolecithin **2** should be most convenient for the O and N-retinoylation of uncharged organic substrates. In this respect the same reaction as described above was carried out on aliphatic **4a** and fatty alcohols **5a** but also on free-hydroxyl anomeric sugars **6a**, **7a** and finally on aromatic amine **8a** or C-protected α-amino-acids **9a**, **10a** (scheme 2).

SCHEME 2



The chemical yields and reaction times are reported into the table and are satisfactory even when compared for those compounds which are available in the literature data.

The one-step procedure which is described here is an easy and efficient method for O and N-retinoylation which avoids the tedious preparation of

retinoylating agents such as retinoyl halides or imidazolides. Moreover, it is a convenient way to be used for coupling of the heavily conjugated and UV sensitive retinoic acid with charged substrates as well as uncharged ones.

TABLE

Substrate	Solvent	Temp.	Time reaction	Product (% yield)
2	$CHCl_3$	61°C	12 h	**3** (50[a])
4a	CH_2Cl_2	R.T	30 min	**4b** (75)
5a	CH_2Cl_2	R.T	30 min	**5b** (78[a])
6a	CH_2Cl_2	R.T	30 min	**6b** (77, 95[b])
7a	CH_2Cl_2	R.T	30 min	**7b** (75)
8a	CH_2Cl_2/DMF	R.T	1 h	**8b** (80, 75[c])
9a	CH_2Cl_2	R.T	30 min	**9b** (80)
10a	CH_2Cl_2	R.T	30 min	**10b** (90, 89[d])

a) The crude material was chromatographed without extraction in order to avoid an untractable emulsion; b) Lit.[8]; c) Lit.[27]; d) Lit.[11]

At last, this method is a highly advisable way for acylation of poorly reactive compounds as we exemplified it by a synthesis of the recently described amide[28] which resulted from the condensation between 2-*trans*-β-ionylidenacetic acid and methyl *p*-aminobenzoate; in this particular case we obtained the desired compound[29] in 85% yield after silica gel column chromatography.

EXPERIMENTAL

Melting points were obtained with a Büchi (capillary) apparatus and were uncorrected. Elemental analyses were performed in microanalysis laboratory of ENSCM (Montpellier). ^1H NMR and ^{13}C NMR were determined with a AC 250

and AC 400 Brucker spectrometer using $CDCl_3$ as solvent. Mass spectra were obtained with a JEOL JMS-DX 300 spectrometer. Thin-layer chromatography (TLC) was performed on silica gel $60F_{254}$ (Merck) with detection by UV light. Column chromatography was performed on silica gel 60. All solvents used for the reactions were anhydrous and all reactions were carried out under inert atmosphere in darkroom.

General procedure: To a stirred solution of retinoic acid (1 eq.), alcohol (or amine) (1.2 eq.) and DMAP (1.2 eq.) in anhydrous methylene chloride (4 cm^3) cooled at 0°C, DCC (1.2 eq.) in methylene chloride (1 cm^3) was added dropwise. The reaction mixture was then stirred at room temperature from 30 min to 12 hrs (see Table) and the reaction was hydrolysed with ice-water and extracted with methylene chloride. The organic layers were washed with brine, dried with anhydrous Na_2SO_4 and the solvents evaporated to dryness under reduced pressure. The crude residue was then purified on a silica gel column chromatography or on preparative TLC.

1-Stearoyl-2-all-trans-retinoyl-glycero-3-phosphorylcholine **3**. According to the general procedure, the crude material was purified on preparative TLC silica gel developed with chloroform-methanol-water (65:25:4) and the phospholipid was obtained as a yellow oil (50% yield); R_f = 0.37 (65:25:4 chloroform-methanol-water). ^1H NMR ($CDCl_3$), δ (ppm) : 6.95-6.85 (m, 1H, H_{11}); 6.25-6.1 (m, 4H, $H_{7,8,10,12}$); 5.7 (s, 1H, H_{14}); 5.15 (m, 1H, C<u>H</u>-); 4.3-4.1 (m, 2H, C<u>H</u>$_2$OCOR); 4.2 (m, 2H, C<u>H</u>$_2$O(choline)); 3.9 (m, 2H, C<u>H</u>$_2$O(glycerol)); 3.65 (m, 2H, C<u>H</u>$_2$N); 3.2 (s, 9H, N(C<u>H</u>$_3$)$_3$); 2.3-1.7 (m, 10H, $H_{16,18,4}$ and C<u>H</u>$_2$CO); 1.6-1.3 (m, 9H, $H_{2,3,19}$

and $C\underline{H}_2CH_2CO$); 1,2 (s, 28H, 14 $C\underline{H}_2$); 0.9 (s, 6H, $H_{16,17}$); 0.8 (t, 3H, $C\underline{H}_3$, *J* 6.8 Hz). ^{13}C NMR ($CDCl_3$), δ (ppm) : 12.85 C_{19}; 13.9 C_{20}; 14.07 $\underline{C}H_3$; 19.15 C_3; 21.71 C_{18}; 22.64 $\underline{C}H_2CH_3$; 24.87 $\underline{C}H_2CH_2CH_3$; 31.87 $\underline{C}H_2CH_2CO$; 33 C_4; 34.17 C_1; 34.3 $\underline{C}H_2CO$; 39.53 C_2; 54.27 $N(\underline{C}H_3)_3$; 59.37 $\underline{C}H_2N$; 63.05 $\underline{C}H_2O$(glycerol); 63.57 $\underline{C}H_2OCOR$; 66.2 $\underline{C}H_2O$(choline); 69.8 $\underline{C}H$; 117.75 C_{14}; 128.8 C_7; 129.45 C_{10}; 129.92 C_5; 131.54 C_{11}; 134.77 C_{12}; 137.18 C_8; 137.57 C_6; 139.87 C_9; 153.88 C_{13}; 166.21 C_{15} and 173.65 $\underline{C}O$. ^{31}P NMR ($CDCl_3$), δ (ppm) : -0.66. FAB^+ (NOBA) : m/z 828 $(M+Na)^+$; 806 $(M+H)^+$. (Found: C, 64.42; H, 10.19; N, 1.46. $C_{46}H_{80}NO_8P.3H_20$ requires C, 64.23; H, 10.08; N, 1.63 %).

All-trans-retinoyl-isopropyl-ester **4b**. According to the general procedure, the crude material was chromatographed initially with hexane and then hexane-ethyl acetate (95:5) as eluting solvents to afford the desired ester as a yellow oil (75% yield); R_f = 0.75 (95:5 hexane:ethyl acetate). ^1H NMR ($CDCl_3$), δ (ppm) : 6.91 (dd, 1H, H_{11}, *J* 11.4, 15 Hz); 6.4-6.1 (m, 4H, $H_{7,8,10,12}$); 5.67 (s, 1H, H_{14}); 4.69 (m, 1H, $C\underline{H}$-); 2.4 (s, 3H, H_{20}); 1.95 (s, 5H, $H_{18,4}$); 1.65 (s, 3H, H_{19}); 1.55 (m, 2H, H_3); 1.4 (m, 2H, H_2); 1.2 (d, 6H, $(C\underline{H}_3)_2$, *J* 6.26 Hz); 0.98 (s, 6H, $H_{16,17}$). ^{13}C NMR ($CDCl_3$), δ (ppm) : 12.87 C_{19}; 14.05 C_{20}; 19.2 C_3; 21.6 C_{18}; 21.98 $(\underline{C}H_3)_2$; 28.93 $C_{16,17}$; 33.08 C_4; 34.23 C_1; 39.57 C_2; 66.75 $\underline{C}H$; 119.15 C_{14}; 128.45 C_7; 129.4 C_{10}; 129.73 C_5; 130.55 C_{11}; 135.24 C_{12}; 137.3 C_8; 137.6 C_6; 139.47 C_9; 152.35 C_{13}; 165 C_{15}. FAB^+ (NOBA) : m/z 343 $(M+H)^+$; 365 $(M+Na)^+$. (Found: C, 80.48; H, 10.25; O, 9.45. $C_{23}H_{34}O_2$ requires C, 80.65; H, 10.00; O, 9.34 %).

1,3-Dipalmitoyl-2-all-trans-retinoylglycerol **5b**. According to the general procedure, the crude material was chromatographed initially with hexane and then

hexane-diethyl ether (95:5) as eluting solvents to afford the desired ester as a yellow oil (78% yield); R_f = 0.56 (95:5 hexane:diethyl ether). ^{1}H NMR ($CDCl_3$), δ (ppm) : 6.95 (m, 1H, H_{11}); 6.25-6 (m, 4H, $H_{7,8,10,12}$); 5.7 (s, 1H, H_{14}); 5.25-5.2 (m, 1H, C<u>H</u>-); 4.3-4.05 (m, 4H, C<u>H</u>$_2$OCOR); 2.3-2.2 (m, 7H, H_{20} and C<u>H</u>$_2$CO); 2-1.9 (m, 5H, $H_{4,18}$); 1.65 (s, 3H, H_{19}); 1.6-1.5 (m, 6H, H_3 and C<u>H</u>$_2$CH$_2$CO); 1.4 (m, 2H, H_2); 1.2 (s, 24H, 12 C<u>H</u>$_2$); 0.95 (s, 6H, $H_{16,17}$); 0.8 (t, 3H, C<u>H</u>$_3$, *J* 6.81 Hz). ^{13}C NMR ($CDCl_3$), δ (ppm) : 12.78 C_{19}; 13.86 C_{20}; 19.08 C_3; 21.6 C_{18}; 33 C_4; 34.12 C_1; 39.48 C_2; 61.48 and 62.02 <u>C</u>H$_2$OCOR; 68 and 68.9 <u>C</u>HOCOR; 117.3 C_{14}; 128.9 C_7; 129.4 C_{10}; 130 C_5; 131.42 C_{11}; 134.67 C_{12}; 137 C_8; 137.31 C_6; 139.9 C_9; 154 C_{13}; 166.4 and 165.9 C_{15}; 173.3 and 172 (2<u>C</u>O). FAB$^+$ (NOBA) : m/z 851 $(M+H)^+$; 552 $[(M-C_{20}H_{27}O_2)^+]$. (Found: C, 77.74; H, 11.06; O, 11.39. $C_{55}H_{94}O_6$ requires C, 77.6; H, 11.13; O, 11.28 %).

All-trans-retinoyl-2,3,4,6-tetra-O-acetyl-D-glucopyranosyl ester **6b**. According to the general procedure, the crude material was chromatographed with diethyl ether-methylene chloride (0 to 10%) as eluting system to afford the desired retinoate as a yellow oil (77% yield); R_f = 0.6 (9:1 methylene chloride:diethyl ether). ^{1}H NMR ($CDCl_3$), δ (ppm): 7.1 (m, 1H, H_{11}); 6.4-6.1 (m, 5H, $H_{1',7,8,10,12}$); 5.8 (s, 1H, H_{14}); 5.3-5.15 (m, 3H, $H_{2',3',4'}$); 5-4.6 (m, 1H, $H_{5'}$); 4.4-4 (m, 2H, $H_{6'}$); 2.35 (s, 3H, H_{20}); 2.15 (s, 3H, H_{18}); 2.1-1.9 (m, 14H, H_4 and 4 C<u>H</u>$_3$CO); 1.7 (s, 3H, H_{19}); 1.6 (m, 2H, H_3); 1.45 (m, 2H, H_2); 1 (s, 6H, $H_{16,17}$). ^{13}C NMR ($CDCl_3$), δ (ppm): 12.87 C_{19}; 14.06 C_{20}; 19.08 C_3; 20.08-20.45 4 <u>C</u>H$_3$CO; 21.67 C_{18}; 28.85 $C_{16,17}$; 33.01 C_4; 34.15 C_1; 39.47 C_2; 61.38 $C_{6'}$; 65.91, 67.52, 68.1, 72.65

$C_{2'}$,$C_{3'}$,$C_{4'}$,$C_{5'}$; 88.19 and 91.15 $C_{1'}$; 116.9 C_{14}; 129 C_7; 129.22 C_{10}; 130.12 C_5; 134.4 C_{11}; 134.33 C_{12}; 137 C_8; 137.51 C_6; 140.7 C_9; 153.3 C_{13}; 164.36-164.5 C_{15}; 169.26-170.6 4x $\underline{C}OCH_3$. FAB^+ (NOBA) : m/z 669 $(M+K)^+$; 653 $(M+Na)^+$; 630 M^+. (Found: C, 64.72; H, 7.48. $C_{34}H_{46}O_{11}$ requires C, 64.75; H, 7.35 %).

All-trans-retinoyl-2,3,4,6-tetra-O-acetyl-D-galactopyranosyl ester **7b**. According to the general procedure, the crude material was chromatographed with diethyl ether-methylene chloride (0 to 10%) as eluting system to afford the desired retinoate as a yellow oil (75% yield); R_f = 0.6 (9:1 methylene chloride:diethyl ether). ^1H NMR ($CDCl_3$), δ (ppm) : 7.1 (m, 1H, H_{11}); 6.4 (d, 1H, $H_{1'}$, *J* 2.7 Hz); 6.35-6.05 (m, 4H, $H_{7,8,10,12}$); 5.8 (s, 1H, H_{14}); 5.6-5.2 (m, 3H, $H_{2',3',4'}$); 4.3 (t, 1H, $H_{5'}$, *J* 6.5 Hz); 4.1 (m, 2H, $H_{6'}$); 2.35 (s, 3H, H_{20}); 2.15 (s, 3H, H_{18}); 2.1-1.9 (m, 14H, H_4 and 4 $C\underline{H}_3CO$); 1.7 (s, 3H, H_{19}); 1.6 (m, 2H, H_3); 1.45 (m, 2H, H_2); 1 (s, 6H, $H_{16,17}$). ^{13}C NMR ($CDCl_3$), δ (ppm) : 12.87 C_{19}; 14.06 C_{20}; 19.08 C_3; 20.08-20.45 4 $\underline{C}H_3CO$; 21.67 C_{18}; 28.85 $C_{16,17}$; 33.01 C_4; 34.15 C_1; 39.47 C_2; 61.38 $C_{6'}$; 65.91, 67.52, 68.1, 72.65 $C_{2'}$,$C_{3'}$,$C_{4'}$,$C_{5'}$; 88.19 and 91.15 $C_{1'}$; 116.9 C_{14}; 129 C_7; 129.22 C_{10}; 130.12 C_5; 134.4 C_{11}; 134.33 C_{12}; 137 C_8; 137.51 C_6; 140.7 C_9; 153.3 C_{13}; 164.36-164.5 C_{15}; 169.26-170.6 4 $\underline{C}OCH_3$. FAB^+ (NOBA) : m/z 669 $(M+K)^+$; 653 $(M+Na)^+$; 630 M^+. (Found: C, 64.67; H, 7.51. $C_{34}H_{46}O_{11}$ requires C, 64.75; H, 7.35 %).

All-trans-4-hydroxyphenylretinamide **8b**. According to the general procedure, the residual solid was purified by column chromatography with diethyl ether-hexane mixture (0 to 20%) as eluting system. the product was obtained as a

yellow powder (80% yield) m.p.160-162°C; R_f = 0.53 (2:8 diethyl ether:hexane). ^{1}H NMR ($CDCl_3$), δ (ppm) : 7.4-6.6 (m, 6H, H_{amide}, H_{11}, H_{arom}); 6.4-6 (m, 5H, $H_{7,8,10,12}$); 5.8 (s, 1H, H_{14}); 2.4 (s, 3H, H_{20}); 1.95 (s, 5H, $H_{18,4}$); 1.65 (s, 3H, H_{19}); 1.55 (m, 2H, H_3); 1.45 (m, 2H, H_2); 1 (s, 6H, $H_{16,17}$). EI$^+$, 30eV : m/z 391 M^+. (Found: C, 79.67; H, 8.53; N, 3.42. $C_{26}H_{33}NO_2$ requires C, 79.76; H, 8.49; N, 3.58 %).

All-trans-N-retinoyl-N$_\varepsilon$-Boc-lysine methyl ester **9b**. A solution of L-N$_\varepsilon$-Boc-lysine methyl ester **9a** was prepared from the hydrochloride (1 eq.) and diisopropylethylamine (1 eq.) in methylene chloride. The general procedure was then applied and the product was obtained as an orange oil after chromatography in diethyl ether-petroleum ether (1:1) as eluting system (80% yield); R_f = 0.26 (1:1 diethyl ether:petroleum ether). ^{1}H NMR ($CDCl_3$), δ (ppm) : 6.95 (dd, 1H, H_{11}, *J* 11, 15 Hz); 6.5-6.1 (m, 5H, H_α, $H_{7,8,10,12}$); 5.8 (s, 1H, H_{14}); 4.6 (m, 3H, O<u>C</u>H$_3$); 3.1 (m, 2H, H_β); 2.4 (s, 3H, H_{20}); 2 (s, 5H, $H_{18,4}$); 1.8 (s, 3H, H_{19}); 1.6 (m, 2H, H_3); 1.5-1.4 (m, 13H, H_2, H_δ, H_γ, H_{tBu}); 1.05 (s, 6H, $H_{16,17}$). ^{13}C NMR ($CDCl_3$), δ (ppm) : 12.81 C_{19}; 13.57 C_{20}; 19.14 C_3; 21.68 C_{18}; 22.34 C_γ; 28.88 C_β; 29.56 $C_{16,17}$; 32 C_4; 33 C_1; 34.22 C_2; 40 C_δ; 51.74 O<u>C</u>H$_3$; 52.33 C_α; 79.11 -C(<u>C</u>H$_3$)$_3$; 120.61 C_{14}; 128.78 C_5; 130.0 C_{11}; 135.36 C_{12}; 137.25 C_8; 137.64 C_6; 138.86 C_9; 149.55 C_{13}; 156.12 <u>C</u>(O)(Boc); 166.7 <u>C</u>(O)OCH$_3$; 173.16 C_{15}. FAB$^+$ (NOBA) : m/z 542 M^+; 543 $(M+H)^+$; 565 $(M+Na)^+$. (Found: C, 70.86; H, 9.40; N, 5.32. $C_{32}H_{50}N_2O_5$ requires C, 70.81; H, 9.28; N, 5.16 %).

All-trans-N-retinoyl-phenylalanine methyl ester **10b**. A solution of L-phenylalanine methyl ester **10a** was prepared from the hydrochloride (1 eq.) and

diisopropylethylamine (1 eq.) in methylene chloride. The general procedure was then applied and the product was obtained as a red oil after chromatography with methylene chloride as eluent (90% yield); R_f= 0.54 (methylene chloride). ^{1}H NMR ($CDCl_3$) δ (ppm) : 7.4-7.1 (m, 5H, H_{arom}); 6.95 (dd, 1H, H_{11}, *J* 11, 15 Hz); 6.4-6 (m, 4H, $H_{7,8,10,12}$); 5.7 (s, 1H, H_{14}); 4.95 (dd, 1H, C<u>H</u>-, *J* 6, 8 Hz); 3.75 (s, 3H, $CO_2C\underline{H}_3$); 3.2 (dd, 2H, -C<u>H</u>$_2$-, *J* 3, 5 Hz); 2.35 (s, 3H, H_{20}); 2 (s, 5H, $H_{18,4}$); 1.8 (s, 3H, H_{19}); 1.6 (m, 2H, H_3); 1.5 (m, 2H, H_2); 1.05 (s, 6H, $H_{16,17}$). ^{13}C NMR ($CDCl_3$) δ (ppm) : 12.95 C_{19}; 13.51 C_{20}; 19.11 C_3; 20.78 C_{18}; 29 $C_{16,17}$; 33 C_4; 34 C_1; 38 C_β; 39.47 C_2; 52.83 O<u>C</u>H$_3$; 52.92 C_α; 118.68 C_{14}; 130-132 C_{arom}, C_{11}, C_5, C_{10}, C_7; 135.25 C_{12}; 135.89 C_8; 137.21 C_6; 137.47 C_9; 149.5 C_{13}; 166.3 <u>C</u>(O)OCH$_3$; 172.18 C_{15}. FAB$^+$ (NOBA) : m/z 461 M^+; 462 $(M+H)^+$; 484 $(M+Na)^+$; 500 $(M+K)^+$; 923 $(2M+H)^+$. (Found: C, 77.79; H, 8.65; N, 3.09. $C_{30}H_{39}NO_3$ requires C, 78.05; H, 8.51; N, 3.03 %).

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