ORIGINAL ARTICLE

Characterization of Methyl and Ethyl Esters of Amino-Acids as Corneal Permeation Enhancers

Rajan Ethiraj Ugandar[1,4], Voleti Vijay Kumar[1], C.Madhusudhana Chetty[1], Dinesh Kumar Sharma[2], Kiran C Nilugal[3], Sandeep Poddar[4]

[1] Department of Pharmaceutics, Faculty of Pharmacy, Santhiram College of Pharmacy, Nandyal, 518501,Kurnool District, Andhra Pradesh, India.
[2] Department of Pharmaceutics, Faculty of Pharmacy, Himalayan Institute of Pharmacy and Research, Dehra Dun,248001, Uttara Khand, India
[3] Department of Pharmacology, School of Pharmacy, Management and Science University, Shah Alam,40100, Malaysia
[4] Lincoln University College, Petaling Jaya, 47301, Malaysia

ABSTRACT

Introduction: Amino acids are important role-playing components in the maintenance of the normal functions of parts of eye like retina and conjunctiva. In the current study the methyl and ethyl esters of amino acids such as lysine, phenyl alanine and valine were used to enhance the corneal permeation of ketorolac tromethamine. **Methods:** The amino-acid esters were coupled with the drug ketorolac tromethamine to obtain the test products and were characterized by various analytical techniques. The characterized test products were used to formulate the test ophthalmic solutions of Ketorolac tromethamine such as KPD-1, KPD-1A, KPD-2, KPD-2A, KPD-3 and KPD-3A with methyl and ethyl esters of corresponding amino-acids. These test products were subjected percentage corneal hydration and to permeation studies by using Franz diffusion cell mounted with freshly isolated goat cornea. **Results:** All the test results were compared with those of the standard Ketorolac tromethamine ophthalmic solution and observed that all the test solutions have exhibited less percentage corneal hydration and enhanced corneal permeation of ketorolac tromethamine. **Conclusion:** From all the results it can be concluded that the NonsteroidalAnti-Inflammatory Ketorolac has enhanced trans-corneal permeation and reduced corneal hydration when formulated with amino acid transporters by the pro-drug approach in ophthalmic solutions as the formulated pro-drugs have revealed high vitreal drug concentration.

Keywords: Amino-acid esters, Characterization, Permeation, Enhancer

Corresponding Author:
Rajan Ethiraj Ugandar, PhD
Email: reugandar@gmail.com
Tel: +918618910930

INTRODUCTION

Ocular administration of drugs is primarily associated with the need to treat ophthalmic disease and is not regarded as a means of gaining systemic drug action. The corneal permeation of a drug from an administered ophthalmic preparation will be around 5% or less while remainder is lost by pre-corneal constrains (1). Pre-corneal constraints include spillage of a drug by overflow, dilution of a drug by tears turnover, naso-lacrimal drainage/systemic drug absorption, non-productive conjunctival drug absorption and enzymatic metabolism and binding of the drug to proteins (2). The most effective method for drug targeting is believed to be to amino acids and peptide transporter as these transporters have the enormous range of substrates and direction of transport from epithelium to endothelium providing a possible task in the permeation of substrate molecule (3). The presence of LATI, ATB0+, ASCTI in cornea are acting as transporters (4, 5) and were involved in the transport of different components to the posterior segment. This has been conceptual and formed the rationale to use the methyl and ethyl esters of amino acids such as arginine, phenylalanine, and alanine to be transporters on parts of the cornea.

MATERIALS AND METHODS

Chemicals & reagents: l-lysine, l-phenyl alanine and l-valine (SAS Chemicals Co., Mumbai). Thionyl chloride (SD fine chem. Ltd., Mumbai). Methanol (SD fine Chemicals Limited). Ethanol (Jiangsu Huaxsi Ltd. China). Ketorolac tromethamine (Unisule Pvt. India Ltd., Sonepat). Tetrahydrofuran (RFCL Limited, New Delhi). Hydroxybenzotriazole (Spectrochem Pvt. Ltd., Mumbai). Dicyclohexylcarbodimide (Spectrochem Pvt. Ltd. Mumbai). Sodium bicarbonate (CDH (P) Ltd., New

Delhi).

Equipments: Heating mantle (Popular, India). Magnetic stirrer (Labfit, India). Rotary film evaporator (Popular, India). UV cabinet (Labfit, India). FTIR (Shimadzu, Singapore). UV Spectrophotometer (Double Beam) (Shimadzu, Japan). Mass Spectrophotometer (Shimadzu, Japan). NMR (Bruker AM-300). Micropipette (P'fact, India). Hot Air Oven (Navyug, India). Electronic Balance (Shimadzu, Japan). pH Meter (Labfit, India). Centrifuge (Remi, India). Melting Point Apparatus (Popular, India). Franz Diffusion Cell/10 ml (Fabricated by Singh Scientific Industries, Ludhiana,India). Desiccator (Popular, India).

Procedure for synthesis of Ketorolac tromethamine-amino acid pro-drugs

One milli mole of Ketorolac tromethamine was dissolved in adequate amount of tetra hydro furan, followed by the addition of one milli mole of Hydroxybenzotriazole solution in Dimethylformamide (DMF). The reaction mixture cooled to zero degree centigrade with constant stirring. Methyl or ethyl ester of amino acid dissolved in DMF and neutralized with triethylamine was added to the above reaction mixture at zero degree centigrade. The above is mixed with dicyclohexylcarbodimide (DCC) and dissolved in THF at zero degree centigrade and the mixture was allowed to reach the room temperature (5). Methyl or ethyl ester of amino acid as well as DCC and hydroxybenzotriazole were used in the same molar concentration as that of Ketorolac tromethamine. The dicyclohexylurea (DCU) was concentrated on a rotary evaporator (6). The oily residue obtained after rotary distillation was dissolved in ethyl acetate, washed with 5% w/v aqueous solution of sodium bicarbonate followed by washing with brine and then washed with 5% w/v citric acid solution and finally rewashed with brine solution (2,3 & 4). The mixture was concentrated on a rotary evaporator to get the pure form of amino acid esters (7).

Characterization

All the synthesized prodrugs of Ketorolac tromethamine were subjected for their characterization by the determination of their FTIR Spectra, H1NMR Spectra and MASS Spectra. The relevant interpretation and their characterization data were represented in Table I.

Permeation studies

The permeation studies were carried out by using a Franz diffusion cell mounted with freshly isolated gat cornea membrane. Permeation studies were carried out to optimize the drug concentration and the pH. The optimized concentration of 0.5% w/v and the optimized pH of 7.4 was considered and adopted throughout the permeation studies of the standard and test samples.

Percentage corneal hydration

At the end of the experiment each cornea was weighed, soaked in methanol for 3-4 minutes, dried at 80° C for overnight and reweighed.

Hydration was calculated by the following formula:

$$\% \text{ Hydration} = (1 - W_d / W_w) \times 100$$

Where, W_d =Weight of dried cornea and
W_w= Weight of wet cornea

Table I: Characterization data of the synthesized test samples of methyl and ethyl esters of amino acids with Ketorolac tromethamine.

Test Samples	FTIR Data	+HNMR Data	Mass Spectra Data FAB) (M+1) +
KPD-1	3299 (NH), 3029 (Aromatic CH), 2936, 2889 (Aliphatic CH), 1720 (C=O str. of ester), 1636 (Amide I), 1530 (Amide II), 1383, 1342 (CH bend, aliphatic), 1255 (C-O str. of ester).	δ 1.28 (d, 3H, —CH_3); 3.32 (m, 1H, 4'-H); 3.56 (m, 1H, —CH); 3.65 (m, 1H, 5'-H); 4.09 (m, 2H, 6'-H); 4.18 (m, 1H, 2'-H); 4.27 (m, 1H, 3'-H); 5.01 (m, 1H, 1'-H); 7.16 (m, 2H, 5'-H and 6'-H); 7.20 (m, 2H, 3"-H and 5"-H); 7.29 (m, 1H, 3-H, 4"-H); 3.04 [(d), 1H] CO-NH, 7.42 (s, 1-H, 2'-H); 7.3 (m, 2-H, 2"-H and 6"-H).	407
KPD-1A	3300 (NH), 3031 (AromaticCH), 2940, 2892 (Aliphatic CH), 1725 (C=O str. of ester), 1640 (Amide I), 1532 (Amide II), 1384, 1348 (CH bend, aliphatic), 1259 (C-O str. of ester).	δ 1.3 (d, 3H, —CH_3); 3.35 (m, 1H, 4'-H); 3.60 (m, 1H, —CH); 3.7 (m, 1H, 5'-H); 4.10 (m, 2H, 6'-H); 4.20 (m, 1H, 2'-H); 4.30 (m, 1H, 3'-H); 5.05 (m, 1H, 1'-H); 7.21 (m, 2H, 5'-H and 6'-H); 7.26 (m, 2H, 3"-H and 5"-H); 7.35 (m, 1H, 3-H, 4"-H); 3.10 [(d), 1H] CO-NH, 7.46 (s, 1-H, 2'-H); 7.6 (m, 2-H, 2"-H and 6"-H).	421
KPD-2	3278.4 (NH), 3022 (Aromatic CH), 2954, 2710 (Aliphatic CH), 1717 (C=O str. of ester), 1646 (Amide I), 1555 (Amide II), 1387, 1412 (CH bend, aliphatic), 1144 (C-O str. of ester).	δ 1.27, 1.28, 1.37, 1.38 (4 s, 12H, ketals); 2.68, 2.77 (m, 2H, 2-H); 3.18 (m, 1H, 1-H); 4.02 (m, 1H, 4'-H); 4.12 (m, 1H, 5'-H); 4.08 (m, 2H, 6'-H); 4.28 (m, 1H, 2'-H); 4.38 (m, 1H, 3'-H); 4.48 (m, 2H, 3-H); 5.23 (m, 1H, 1'-H); 5.78 (d, 1H, 7-H); 6.60 (d, 1H, 6-H); 7.33 (m, 2H, 2,6-Ph); 7.43 (m, 1H, 4-Ph); 7.71 (m, 2H, 3,5-Ph).	414
KPD-2A	3280.6 (NH), 3025 (Aromatic CH), 2955, 2712 (Aliphatic CH), 1720 (C=O str. of ester), 1650 (Amide I), 1560 (Amide II), 1392, 1418 (CH bend, aliphatic), 1145 (C-O str. of ester).	δ 1.29, 1.30, 1.39, 1.40 (4 s, 12H, ketals); 2.70, 2.80 (m, 2H, 2-H); 3.20 (m, 1H, 1-H); 4.03 (m, 1H, 4'-H); 4.15 (m, 1H, 5'-H); 4.10 (m, 2H, 6'-H); 4.30 (m, 1H, 2'-H); 4.40 (m, 1H, 3'-H); 4.50 (m, 2H, 3-H); 5.25 (m, 1H, 1'-H); 5.80 (d, 1H, 7-H); 6.70 (d, 1H, 6-H); 7.35 (m, 2H, 2,6-Ph); 7.45 (m, 1H, 4-Ph); 7.75 (m, 2H, 3,5-Ph).	428
KPD-3	3359 (NH), 3871.37 (NH str of indole ring, 3015 (Aromatic CH), 2912, 2841 (Aliphatic CH), 1714 (C=O str of ester), 1627 (Amide I), 1537 (Amide II), 1423, 1364 (CH bend, aliphatic), 1219 (C-O str of ester).	δ 1.2 (d, 3H, —CH_3); 3.33 (m, 1H, 4'-H); 3.58 (m, 1H, —CH); 3.5 (m, 1H, 5'-H); 4.08 (m, 2H, 6'-H); 4.18 (m, 1H, 2'-H); 4.28 (m, 1H, 3'-H); 5.03 (m, 1H, 1'-H); 7.20 (m, 2H, 5'-H and 6'-H); 7.24 (m, 2H, 3"-H and 5"-H); 7.30 (m, 1H, 3-H, 4"-H); 7.43 (s, 1-H, 2'-H); 7.3 (m, 2-H, 2"-H and 6"-H).	498
KPD-3A	3360 (NH), 3873.37 (NH str of indole ring, 3020 (Aromatic CH), 2915, 2845 (Aliphatic CH), 1720 (C=O str of ester), 1634 (Amide I), 1545 (Amide II), 1423, 1375 (CH bend, aliphatic), 1221 (C-O str of ester).	δ 1.3 (d, 3H, —CH_3); 3.35 (m, 1H, 4'-H); 3.60 (m, 1H, —CH); 3.7 (m, 1H, 5'-H); 4.10 (m, 2H, 6'-H); 4.20 (m, 1H, 2'-H); 4.30 (m, 1H, 3'-H); 5.05 (m, 1H, 1'-H); 7.21 (m, 2H, 5'-H and 6'-H); 7.26 (m, 2H, 3"-H and 5"-H); 7.35 (m, 1H, 3-H, 4"-H); 7.46 (s, 1-H, 2'-H); 7.6 (m, 2-H, 2"-H and 6"-H).	512

KPD-1:Ketorolac tromethamine pro drug with methyl ester of l-lysine
KPD-1A:Ketorolac tromethamine pro drug with ethyl ester of l-lysine
KPD-2:Ketorolac tromethamine pro drug with methyl ester of l-phenylalanine
KPD-2A:Ketorolac tromethamine pro drug with ethyl ester of l-phenylalanine
KPD-3:Ketorolac tromethamine pro drug with methyl ester of l-valine
KPD-3A:Ketorolac tromethamine pro drug with ethyl ester of l-valine

RESULTS

Characterization of the synthesized pro-drugs of Ketorolac tromethamine

The synthesized prodrugs KPD-1. KPD-1A, KPD-2, KPD-2A, KPD-3 and KPD-3A were subjected for their characterization by the determination of their melting points, RF values, FTIR Spectra, H1NMR Spectra, MASS Spectra, and all the observed values confirmed their characteristics. The optimum drug concentration was found to be 0.5% W/V and the optimum pH was found to be 7.4 with maximum permeation during the permeation studies carried out by using Franz diffusion cell mounted with freshly isolated goat cornea membrane (Table II). The percentage permeability of the drug with all pro-drugs (KPD-1. KPD-1A, KPD-2, KPD-2A, KPD-3 and KPD-3A) prepared with the methyl and ethyl esters of all the three amino acids were compared with that of standard. All solutions prepared with pro-drugs shown better permeation than the standard. The results were represented in Fig. 1 and Fig. 2.

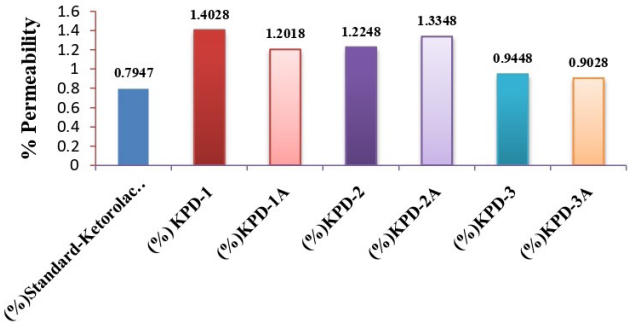

Figure 1: Comparison of percentage permeability of standard Ketorolac tromethamine with the percentage permeability of pro-drugs (KPD-1. KPD-1A, KPD-2, KPD-2A, KPD-3 and KPD-3A) at optimum pH 7.4

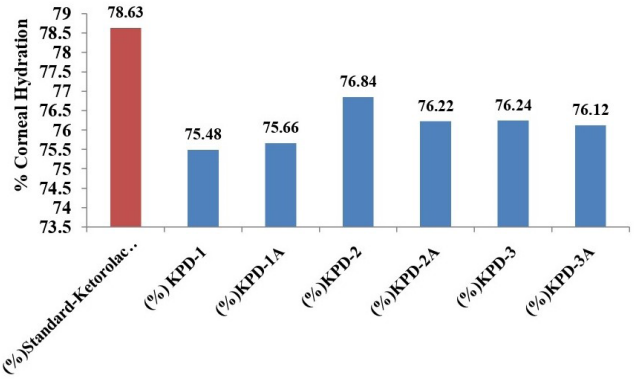

Figure 2: Comparison of percentage corneal hydration of standard Ketorolac tromethamine with the percentage corneal hydration of pro-drugs (KPD-1. KPD-1A, KPD-2, KPD-2A, KPD-3 and KPD-3A) at optimum pH 7.4

DISCUSSION

The amino acids selected for the synthesis of the prodrugs of Ketorolac were: L-lysine, L-phenylalanine, and L-valine. The synthesized prodrugs of Ketorolac were coded as KPD-1 and KPD-1A for the methyl and ethyl esters of Ketorolac with L-Lysine respectively, KPD-2 and KPD-2A for the methyl and ethyl esters of Ketorolac with L-phenyl alanine respectively and KPD-3 and KPD-3A for the methyl and ethyl esters of Ketorolac

Table II: Comparison of percentage permeability and the comparison of percentage corneal hydration of the standard Ketorolac tromethamine with synthesized test samples of methyl and ethyl esters of amino acids with Ketorolac tromethamine

% Permeability standard and samples at different pH				% Corneal hydration		
pH	**Standard**	**KPD-1**	**KPD-1A**	**Standard**	**KPD-1**	**KPD-1A**
6.8	0.4236±0.0846	0.6226±0.1102	0.6016±0.1024	79.87±0.546	77.87±0.536	77.48±0.436
7.0	0.5642±0.0332	0.7246±0.0443	0.7006±0.0443	79.64±2.028	77.86±2.128	77.44±2.248
7.2	0.6424±0.2102	0.8947±0.2102	0.8742±0.1102	79.84±1.212	76.66±1.212	76.24±1.212
7.4*	**0.7947±0.2102**	**1.4028±0.2201**	**1.2018±0.1201**	**78.63±1.212**	**75.48±1.212**	**75.66±1.212**
7.6	0.6232±0.2462	0.8484±0.0624	0.8263±0.0421	81.34±1.046	78.26±1.046	77.68±1.046
pH	**Standard**	**KPD-2**	**KPD-2A**	**Standard**	**KPD-2**	**KPD-2A**
6.8	0.4236±0.0846	0.5816±0.1246	0.6026±0.2346	79.87±0.0332	78.67±0.236	76.46±0.524
7.0	0.5642±0.0332	0.6106±0.0213	0.6606±0.1214	79.64±2.128	78.46±1.248	77.22±1.212
7.2	0.6424±0.2102	0.7642±0.0142	0.7842±0.0142	79.84±0.2102	79.84±1.236	77.82±1146
7.4*	**0.7947±0.2102**	**1.2248±0.2204**	**1.3348±0.1404**	**78.63±1.046**	**76.84±2.242**	**76.22±1.142**
7.6	0.6232±0.2462	0.8168±0.0221	0.8264±0.0128	81.34±0.526	82.26±1.068	80.12±1.242
pH	**Standard**	**KPD-3**	**KPD-3A**	**Standard**	**KPD-3**	**KPD-3A**
6.8	0.4236±0.0846	0.5444±0.1326	0.5212±0.1426	79.87±0.546	78.24±0.224	78.16±0.142
7.0	0.5642±0.0332	0.6008±0.1124	0.5984±0.1142	79.64±2.028	78.48±1.212	77.26±1.547
7.2	0.6424±0.2102	0.6812±0.0212	0.6422±0.0212	79.84±1.212	78.64±1.426	79.14±1.146
7.4*	**0.7947±0.2102**	**0.9448±0.1204**	**0.9028±0.1024**	**78.63±1.212**	**76.24±1.121**	**76.12±1.822**
7.6	0.6232±0.2462	0.8064±0.0102	0.7864±0.0102	81.34±1.046	81.98±1.248	82.44±1.122

* Optimum pH
KPD-1:Ketorolac tromethamine pro drug with methyl ester of l-lysine
KPD-1A:Ketorolac tromethamine pro drug with ethyl ester of l-lysine
KPD-2:Ketorolac tromethamine pro drug with methyl ester of l-phenylalanine
KPD-2A:Ketorolac tromethamine pro drug with ethyl ester of l-phenylalanine
KPD-3:Ketorolac tromethamine pro drug with methyl ester of l-valine
KPD-3A:Ketorolac tromethamine pro drug with ethyl ester of l-valine

with L-valine respectively. The synthesized and purified pro-drugs were subjected to characterization by various physical as well as analytical determinations for the conformational analysis. All the synthesized prodrugs were subjected to several characterization procedures. The characterizations of all the synthesized prodrugs were confirmed by determining the Melting points, TLC and instrumental analytical techniques like FTIR, +HNMR and Mass spectroscopy.

The Melting points and the Rf values obtained by TLC of all the pro-drugs of Ketorolac confirmed their characteristics. The analytical reports such as FTIR spectra, +HNMR and Mass spectroscopy confirmed all the characteristic peaks of the synthesized prodrugs of Ketorolac KPD-1 and KPD-1A, KPD-2 and KPD-2A, KPD-3 and KPD-3A. The ex-vivo trans-corneal permeation studies of Ketorolac tromethamine in aqueous solution and the solutions prepared with the pro-drugs of Ketorolac were carried out. The effect of concentration of Ketorolac aqueous solution on permeation of drug through excised goat cornea was studied with an optimized pH 7.4 using solutions of different concentrations such as 0.5%, 0.1%, 0.15% and 2.0% w/v. It was observed that the 0.5% w/v solution shown a maximum (0.6270%) percentage permeation. Hence a concentration of 0.5% w/v and the concentrations of pro-drugs equivalent to 0.5% w/v were considered as optimum concentrations for the formulations of Ketorolac and for the pro-drugs respectively.

The percentage permeability studies were carried out for the standard Ketorolac tromethamine and all test KPD-1,KPD-1A, KPD-2, KPD-2A, KPD-3 and KPD-3A. On comparison all the percentage permeability of all prodrugs was found to be more than that of the standard. The percentage permeability of standard Ketorolac tromethamine solution (0.5%w/v) with the percentage permeability of the solutions of Ketorolac pro-drugs KPD-1 and KPD-1A at pH 6.8, 7.0, 7.2, 7.4 and 7.6, were shown and the percentage permeability values of the pro-drugs were found to be predominantly high.

On comparison of percentage corneal hydration of standard Ketorolac tromethamine solution (0.5%w/v) with the corneal hydration of the solutions of Ketorolac pro-drugs KPD-1 and KPD-1A at different pH 6.8, 7.0, 7.2, 7.4 and 7.6, it was observed that the percentage corneal hydration of the prodrugs KPD-1 and KPD-1A were found to be less than that of the standard at all pH conditions. Among all the comparative studies it was observed that the percentage permeabilities of prodrugs were high and percentage corneal hydration values of the pro-drugs were less when compared with those of standard Ketorolac tromethamine solution.

CONCLUSION

The methyl and ethyl esters of the selected amino acids were successfully synthesized and were further used for coupling with the pure drug such as Ketorolac tromethamine to yield the amino acid prodrugs of Ketorolac. The synthesizedprodrugs were characterized by various spectral studies such as FTIR, NMR, and Mass spectrometry. All the characterization results of the synthesizedprodrugs of Flurbiprofen and Ketorolac were satisfactory and successful.

From all the results it can be concluded that the NonsteroidalAnti-Inflammatory Ketorolac has enhanced trans-corneal permeation and reduced corneal hydration when formulated with amino acid transporters by the pro-drug approach in ophthalmic solutions as the formulated pro-drugs have revealed high vitreal drug concentration. Further studies can be carried out with different NonsteroidalAnti-Inflammatory Drugs with different amino acids by using the prodrug approach.

ACKNOWLEDGMENTS

The authors acknowledge all who were involved directly and indirectly as professional, technical and non technical support in the research work.

REFERENCES

1. Vyas etal, Current Pharmaceutical Design, 2009; 15(23): 2727.
2. Ugandar RE et al., Flurbiprofen Ophthalmic Solutions Targeting The Posterior Segment Of Eye: An Ex-Vivo Study And A Novel Prodrug Approach On Excised Goat Cornea. European Journal of Pharmaceutical and Medical Research, 2017, 4(8), 400-418.
3. Ravindar Dharavath, Rashmi Patil & Vilas Gaikar "Adsorption of Acidic Impurities from Organic Esters Using Basic Ion Exchange Resins as Functionalized Polymers", Separation Science and Technology, 2005; 40:14, 2947-2962, doi: 10.1080/01496390500333236.
4. Andrew R. Collins. "Comparison of effects of fostriecin, novobiocin, and camptothecin, inhibitors of DNA topoisomerases, on DNA replication and repair in human cells", Nucleic Acids Research, 1990:18(4),1007–1013, https://doi.org/10.1093/nar/18.4.1007.
5. Bringmann A, Grosche A, Pannicke T, Reichenbach A. GABA and Glutamate Uptake and Metabolism in Retinal Glial (Mьller) Cells. Frontiers in Endocrinology, 2013; 4: 48. doi:10.3389/fendo.2013.00048.
6. Araьjo JR, Correia-Branco A, Ramalho C, et al. l-Methionine Placental Uptake: Characterization and Modulation in Gestational Diabetes Mellitus. Reproductive Sciences, 2013; 20(12): 1492-1507. doi:10.1177/1933719113488442.
7. Li, J.; Sha, Y. A Convenient Synthesis of Amino Acid Methyl Esters. Molecules, 2008; 13: 1111-1119.