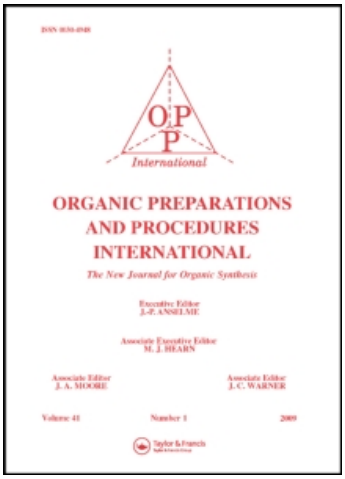

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AN IMPROVED SYNTHETIC PROCESS FOR YF-6, A PROMISING ANTI-HBV DRUG CANDIDATE

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AN IMPROVED SYNTHETIC PROCESS FOR YF-6, A PROMISING ANTI-HBV DRUG CANDIDATE

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(12/14/07)

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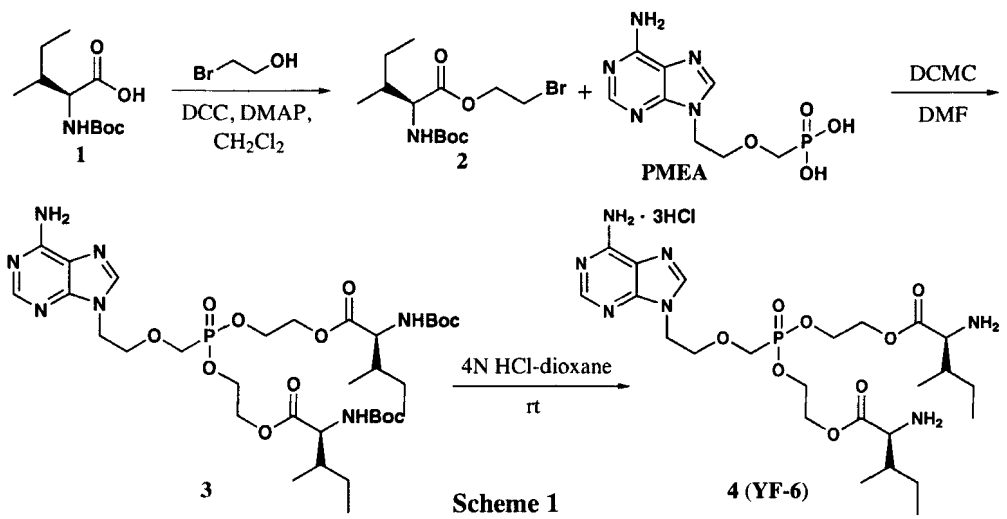
YF-6 (**4**), an ethylene glycol-linked L-amino acid conjugate of 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA), is a promising anti-HBV drug candidate which has been reported from our laboratory, albeit in less than 2% overall yield (*Scheme 1*).[1] Condensation of



Scheme 1

N-Boc-L-isoleucine (**1**) with 2-bromoethanol in the presence of 1,3-dicyclohexylcarbodimide (DCC) and 4-(dimethylamino pyridine (DMAP) provided N-Boc-L-isoleucine 2-bromoethyl ester (**2**) in 75% yield. Subsequent coupling of **2** with PMEA in the presence of N,N'-dicyclohexyl-4-morpholinecarboxamidine (DCMC) produced compound **3** in only 5% yield. Removal of the N-Boc protecting group of compound **3** was achieved with 4N hydrochloride in dioxane to afford **YF-6** in 90% yield. It is apparent that the coupling reaction of PMEA with compound **2** is a limiting step for the whole synthetic process of **YF-6**. The low yield of the coupling step was probably due to the decreased reactivity of the bromoethyl ester **2** and its instability under base conditions.[2] In order to satisfy the urgent need of further investigations for the *in vivo* activity and toxicity studies, it became necessary to explore a good and reliable process for providing multigram quantities of **YF-6** in relatively high yield. Thus, we herein report reaction conditions attempted for the key coupling step and further discuss the effects of solvents, bases and temperature upon the yields (*Table 1*).

Table 1. Conditions Attempted for the Coupling Reaction of Compound **2** with PMEA[a]

Entry	Base	Solvent	Time	Yield[b]
1	DCMC	DMF	rt 48 h, 80°C 8 h	5%
2	DCMC+TMG	DMF	rt 48 h, 80°C 6 h	8%
3	DCMC+TEA	DMF	rt 48 h, 80°C 6 h	9%
4	DCMC+DMAP	DMF	rt 48 h, 80°C 6 h	5%
5	DCMC+Cs_2CO_3	DMF	rt 48 h, 80°C 6 h	14%
6	DCMC+DBU	DMF	rt 48 h, 80°C 6 h	30%
7	DCMC+DBU	DMF	rt 0.5 h, 80°C 15 h	43%
8	DCMC+DBU	Pyridine	rt 0.5 h, 80°C 15 h	0%
9	DCMC+DBU	DMSO	rt 0.5 h, 80°C 15 h	0%
10	DCMC+DBU	NMP	rt 0.5 h, 80°C 15 h	45%

a) All reactions were carried out at the same molar ratio, PMEA:DCMC:base = 1:2:2; b)Yields based on PMEA

Initially, the reaction was carried out with 2 equiv of DCMC and 2 equiv of base in DMF. Addition of DCMC could increase the solubility of PMEA in DMF and efficiently promote the coupling reaction of PMEA and **2**. Several bases such as 1,1,3,3-tetramethylguanidine (TMG), DMAP, TEA, Cs_2CO_3 and DBU were selected. It was noted that the presence of the hindered bases, such as Cs_2CO_3 and DBU, could facilitate the coupling reaction smoothly. Other bases, TMG and TEA, resulted in a low yield of compound **3**. In early studies, the reaction was carried out in two temperature stages: first at room temperature for 48 h and then at 80°C for 6 h. It was observed that the best yield was obtained when the reaction was carried out at 80°C for 15 h, suggesting that prolonging reaction exposure temperature at 80°C is beneficial. At higher temperatures, extensive side-reactions occurred. With regard to the solvent effects, N,N-

dimethylformamide (DMF), pyrimidine, dimethyl sulfoxide (DMSO) and N-methyl-2-pyrrolidone (NMP) were selected as solvents. Among these solvents, using pyrimidine or DMSO as solvents afforded poor yields of compound **3**. Better results were obtained when the reaction was carried out in polar aprotic solvents DMF and NMP. In view of the cost and ease of workup, we chose the DCMC/DBU/DMF system as the final optimized conditions to produce compound **3** in above 40% yield.

In conclusion, **YF-6** was prepared starting from N-Boc-L-isoleucine in three steps in 28% overall yield. To circumvent the low yield of the coupling reaction of PMEA with compound **2**, the survey of temperature, bases and solvents was examined and the DCMC/DBU/DMF system was explored as the best reaction conditions to provide compound **3** in no less than 40% yield. The synthesis is efficient and has been scaled up to prepare 100g of **YF-6**.

EXPERIMENTAL SECTION

^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer using tetramethylsilane as an internal standard for ^{1}H and ^{13}C NMR and 85% H_3PO_4 as an external standard for ^{31}P NMR. Low resolution mass spectra and high resolution mass spectra were obtained using a Kratos MS80 mass spectrometer. Elemental analysis for carbon, hydrogen, and nitrogen were determined on a Vario EL elemental analyzer. Flash chromatography was carried out on silica gel (200-300mes) and chromatographic solvent proportions were expressed on a volume:volume basis. N,N-Dimethylformamide, dichloromethane and triethylamine were dried by distillation under vacuum from calcium hydride. Other commercially available chemicals and solvents were used without further purification. 2-Bromoethanol[3] and PMEA[4] were prepared by literature methods.

N-Boc-L-isoleucine 2-Bromoethyl Ester (2).- To a mixture of N-Boc-L-isoleucine (20.0 g, 83.2 mmol), 2-bromoethanol (13.6 g, 108.8 mol) and DMAP (12.1 g, 99.2 mol) in CH_2Cl_2 (600 mL) was added in portions DCC (20.5 g, 99.4 mmol) at room temperature. The reaction mixture was stirred at room temperature for 24 h. The N, N'-dicyclohexylurea was filtered off and the filtrate was concentrated *in vacuo* to give compound **2** (21.2 g, 75.2%) as a colorless liquid that was pure enough (90-95%) for use in the next step. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.99 (d, J = 8.15 Hz, 1H, COC<u>H</u>NH), 4.48-4.37 (t, J = 6.07 Hz, 2H, $BrCH_2$), 4.28 (m, 1H, NH), 3.52 (t, J = 6.07 Hz, 2H, CH_2OCO), 1.87 (m, 1H, $CH_3C\underline{H}CH_2$), 1.42 (s, 9H, $C(CH_3)_3$), 1.22-1.27 (m, 2H, $CHC\underline{H}_2CH_3$), 0.94-0.87 (m, 6H, 2 x CH_3).

9-[2-(Phosphonomethoxy)ethyl]adenine bis[(N-Boc-L-isoleucyloxy)ethyl] Ester (3).- To a solution of PMEA (2.0 g, 7.3 mmol) in anhydrous DMF (30 mL) at room temperature were added DCMC (4.2 g, 14.4 mmol), bromide **2** (4.8 g, 14.4 mmol) and DBU (2.2 mL, 14.4 mmol). The heterogeneous mixture became homogeneous after 30min and was stirred at 80°C for 15 h. The insolubles were filtered off and the filtrate was concentrated *in vacuo*. The resultant residue was partitioned between water (100 mL) and ethyl acetate (100 mL), and the aqueous layer was extracted with ethyl acetate (1 x 100 mL). The organic phase was concentrated *in vacuo*. The residue was purified by silica gel chromatography, eluting with 5% MeOH/CH_2Cl_2 to provide **3**

(2.5 g, 42.7%) as a very hygroscopic white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 (s, 1H, 2'-H), 7.96 (s, 1H, 8'-H), 5.87 (brs, 2H, NH_2), 5.21-5.40 (m, 2H, COCHNH), 4.39 (t, J = 5.32Hz, 2H, NCH_2), 4.10-4.37 (m, 8H, 2 x OCH_2CH_2O), 3.93 (m, 2H, OCH_2), 3.81 (d, J = 8.41Hz, 2H, OCH_2PO), 1.84 (m, 2H, 2 x CH_3CHCH_2), 1.42 (s, 18H, 2 x $C(CH_3)_3$), 1.05-1.26 (m, 4H, 2 x CH_2CH_3), 0.85-0.91 (m, 12H, 4 x CH_3); EI-MS (m/z): 788 (M^+, 12), 178 (79), 163 (100), 57 (19).

Anal. Calcd for $C_{34}H_{58}N_7O_{12}P$: C: 51.83, H: 7.42, N: 12.45. Found: C: 51.79, H: 7.59, N: 12.16. Its elemental analysis is in agreement with data given in the literature.[1]

9-[2-(Phosphonomethoxy)ethyl]adenine bis[(L-Isoleucyloxy)ethyl] Ester (YF-6).- To a solution of compound **3** (2.5 g, 3.2 mmol) in dry 1,4-dioxane (10 mL) was added 4N hydrochloride dioxane solution (4.96 mL, 19.8 mmol). The reaction mixture was stirred at room temperature for 3 h. After the solvent was removed *in vacuo*, the resultant residue was dissolved in ethanol (5 mL) and the solution was stirred at 0°C for 15 min. Ethyl acetate (2 mL) was added to the solution and stirred at 0°C for an additional 30 min. The resultant product was collected and washed quickly with ethyl acetate. The resultant white solid was dissolved in water (5 mL) and then dried by lyophilization to produce **YF-6** (1.86 g, 90%) as a very hygroscopic white solid. ^{1}H NMR (400 MHz, CD_3OD): δ 8.32 (s, 1H, 2'-H), 7.96 (s, 1H, 8'-H), 5.87 (brs, 2H, NH_2), 5.21-5.40 (m, 2H, COCHNH), 4.39 (t, J = 5.3 Hz, 2H, NCH_2), 4.10-4.37 (m, 8H, 2 x OCH_2CH_2O), 3.93 (m, 2H, OCH_2), 3.81 (d, J = 8.41Hz, 2H, OCH_2PO), 1.84 (m, 2H, 2 x CH_3CHCH_2), 1.05-1.26 (m, 4H, 2 x CH_2CH_3), 0.85-0.91 (m, 12H, 4 x CH_3); EI-MS (m/z): 588(M^+, 11), 86 (100). ^{13}C NMR (100 MHz ,CD_3OD): δ 170.3 (2C), 152.2, 151.0, 146.6, 145.8, 120.2, 72.7, 66.6 (2C), 66.1 (2C), 65.1, 58.8 (2C), 45.5, 38.3 (2C), 27.2 (2C), 15.0 (2C), 12.6 (2C). ^{31}P (160 MHz , CD_3OD): δ 24.0.

Anal. Calcd for $C_{24}H_{42}N_7O_8P \cdot 3HCl \cdot 2H_2O$: C: 39.31, H: 6.68, N: 13.37.
Found: C: 39.17, H: 6.91, N: 13.16.

Its elemental analysis is in agreement with data given in the literature.[1]

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