JOC*Note*

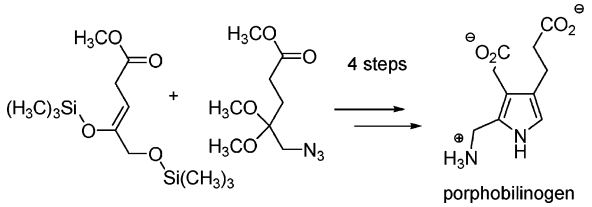
Facile Synthesis of a "Ready to Use" Precursor of Porphobilinogen and Its Amino Acid Derivatives

Carole Pissot Soldermann, Ramakrishnan Vallinayagam, Manuel Tzouros, and Reinhard Neier*

Institut de Chimie, Université de Neuchatel, Rue Emile-Argand 11, PO 158, CH-2009 Neuchatel, Switzerland

reinhard.neier@unine.ch

Received October 29, 2007



A practical synthesis of porphobilinogen based on the biosynthetic mechanism is described. The crossed Mukayiama aldol reaction is the key step creating the central carbon−carbon bond between the two protected forms of 5-aminolevulinic acids. The optimized sequence gives a crystalline, storable precursor, which can be transformed in high yield into porphobilinogen and bioconjugates thereof. The enzymatic hydrolysis of the precursor produces porphobilinogen in quantitative yield.

The simplicity of Nature's biosynthetic pathways has been a motivation for the synthesis chemist since Sir Robert Robinson's synthesis of tropinone.[1] The biomimetic approach has been applied to analyze[2a,b] and understand[2c−e] the structures of natural products. Inspired by the biosynthetic pathways, complicated natural products have been synthesized efficiently.[3] We report the expedient synthesis of porphobilinogen (PBG), imitating the enzymatic mechanism postulated by Shemin.[4] The elegant biosynthesis of the macrocylic structure of the "pigments of life"[5] is highly convergent.[6]

Biosynthetic precursors of the "pigments of life" are increasingly used in medicinal and agrochemical applications.[7] Several syntheses of PBG have been reported recently in view of these applications.[4c,8] Herein, we report a practical synthesis of PBG whose retrosynthesis is based on a biomimetic approach.

Despite its deceptive simplicity, PBG remains difficult to synthesize and to isolate.[8,9] A biomimetic synthesis of a protected form of PBG has been previously developed in our group.[10] In the first-generation synthesis (Scheme 1), the *N*-phthalimido-protected PBG was obtained in a three-step procedure. The crossed Mukaiyama aldol reaction[11] between the regioselectively formed silyl enol ether of methyl 5-phtalimidolevulinate and the monocyanide of succinic acid monomethyl ester created the critical C−C bond. The reduction of the acetylated cyanohydrin produced the crucial methyl amino group, which triggered the formation of the pyrrole ring.[10] The final deprotection had been reported in the literature.[12] The yield of this step is unsatisfactory. We could not improve this step despite considerable efforts.[13]

Replacing the phthalimido protecting group by the tetrachlorophthalimido group allows using milder conditions for the deprotection step.[14] In the hope that this strategy could also be applied to our problem, we synthesized tetrachlorophthalimido-protected 5-aminolevulinic acid and tetrachlorophthalimido aminoacetone as model compounds. Both compounds could be transformed into the corresponding silyl enol ethers using the Miller methodology (Scheme 2).[15]

With these precursors in hand we decided to determine the scope and limitation of the directed Mukaiyama crossed aldol reaction using acyl cyanides as electrophiles.[16] A systematic study was undertaken with the goal to clarify the reactivity constraints for this critical reaction step (Table 1). The crucial C−C bond could be formed to give modest to excellent yields of the corresponding β-keto-cyanohydrins provided the nucleo-

(1) (a) Robinson, R. *J. Chem. Soc.* **1917**, *111*, 876−99. (b) Robinson, R. *J. Chem. Soc.* **1936**, 1079−90.

(2) (a) Eschenmoser, A.; Arigoni, D. *Helv. Chim. Acta* **2005**, *88*, 3011−50. (b) Eschenmoser, A.; Ruzicka, L.; Jeger, O.; Arigoni, D. *Helv. Chim. Acta* **1955**, *38*, 1890−904. (c) Eschenmoser, A. *Angew. Chem., Int. Ed. Engl.* **1988**, *100*, 5−40. (d) Eschenmoser, A.; Loewenthal, E. *Chem. Soc. Rev.* **1992**, *21*, 1−16. (e) Eschenmoser, A. *Science* **1999**, *284*, 2118−24.

(3) (a) Nicolaou, K. C.; Montagnon, T.; Snyder, S. A. *Chem. Commun.* (*Cambridge*) **2003**, 551−64. (b) de la Torre Maria, C.; Sierra Miguel, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 160−81.

(4) (a) Nandi, D. L.; Shemin, D. *J. Biol. Chem.* **1968**, *243*, 1236−42. (b) Stauffer, F.; Zizzari, E.; Soldermann-Pissot, C; Faurite, J.-P.; Neier R. *Chimia* **2001**, *55*, 314−9. (c) Neier, R. In *Advances in Nitrogen Heterocycles*; Moody, C. J., Ed.; JAI Press: New York, 1996; Vol. 2, pp 35−146.

(5) (a) Battersby, A. R. *Science* **1994**, *264*, 1551−7. (b) Battersby, A. R. *Nat. Prod. Rep.* **2000**, *17*, 507−26.

(6) (a) Battersby, A. R. *Nat. Prod. Rep.* **1987**, *4*, 77−87. (b) Kraeutler, B. *Chimia* **1987**, *41*, 277−92.

(7) (a) Fukuda, H.; Casas, A.; Batlle, A. *Int. J. Biochem. Cell Biol.* **2005**, *37*, 272−6. (b) Fotinos, N.; Campo, M. A.; Popowycz, F.; Gurny, R.; Lange, N. *Photochem. Photobiol.* **2006**, *82*, 994−1015. (c) Rebeiz, C. A.; Montazer-Zouhoor, A.; Mayasich, J. M.; Tripathy, B. C.; Wu, S. M.; Rebeiz, C. C. *ACS Symp. Ser.* **1987**, *339*, 295−328. (d) Nandihalli, U. B.; Duke, S. O. *ACS Symp. Ser.* **1993**, *524*, 62−78.

(8) (a) Ahmed, R.; Leeper, F. J. *Org. Biomol. Chem.* **2003**, *1*, 21−3. (b) Jacobi, P. A.; Li, Y. *J. Am. Chem. Soc.* **2001**, *123*, 9307−12. (c) Adamczyk, M.; Reddy, R. E. *Tetrahedron* **1996**, *52*, 14689−700. (d) De Leon, C. Y.; Ganem, B. *J. Org. Chem.* **1996**, *61*, 8730−1.

(9) (a) Adamczyk, M.; Reddy, R. E. *Tetrahedron Lett.* **1995**, *36*, 9121−4. (b) Neier, R. *J. Heterocycl. Chem.* **2000**, *37*, 487−508.

(10) (a) Chaperon, A. R.; Engeloch, T. M.; Neier, R. *Angew. Chem., Int. Ed.* **1998**, *37*, 358−60. (b) Bertschy, H.; Meunier, A.; Neier, R. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 777−8. (c) Neier, R.; Soldermann-Pissot, C. Process for the preparation of porphobilinogen, Eur. Pat. Appl. 1357110, 2003.

(11) Mukaiyama, T. The Directed Aldol Reaction. In *Organic Reactions*; Dauben, G. W., Ed.; Wiley & Sons: New York, 1982; pp 203−331.

(12) (a) Kenner, G. W.; Smith, K. M.; Unsworth, J. F. *J. Chem. Soc., Chem. Commun.* **1973**, 43−4. (b) Kenner, G. W.; Rimmer, J.; Smith, K. M.; Unsworth, J. F. *J. Chem. Soc., Perkin Trans. 1* **1977**, 332−40.

(13) Engeloch, T. These de doctorat, University of Neuchâtel, 1998.

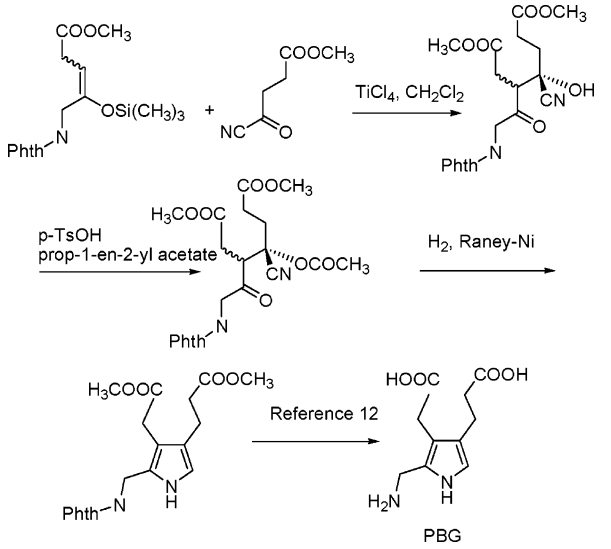
(14) (a) Debenham, J. S.; Madsen, R.; Roberts, C.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117*, 3302−3. (b) Debenham, J. S.; Rodebaugh, R.; Fraser-Reid, B. *J. Org. Chem.* **1997**, *62*, 4591−4600.

(15) Miller, R. D.; McKean, D. R. *Synthesis* **1979**, 730−2

(16) (a) Tang, Q.; Sen, S. E. *Tetrahedron Lett.* **1998**, *39*, 2249−52. (b) Shen, Z.; Li, B.; Wang, L.; Zhang, Y. *Tetrahedron Lett.* **2005**, *46*, 8785−8. (c) Kraus, G. A.; Shimagaki, M. *Tetrahedron Lett.* **1981**, *22*, 1171−4.

10.1021/jo702319n CCC: $40.75
Published on Web 12/15/2007

SCHEME 1. First-Generation Synthesis of *N*-Phthalimido-Protected PBG Using the Mukaiyama Aldol Reaction[10]



SCHEME 2. Synthesis of *N*-Tetracholorphthalimido Amino Acetone and Aminolevulinic Acid and Their Silyl Enol Ethers

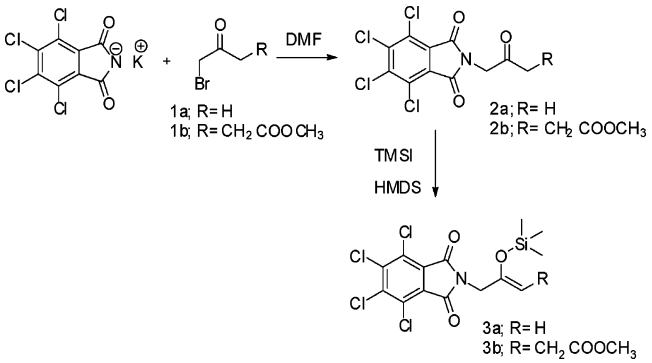
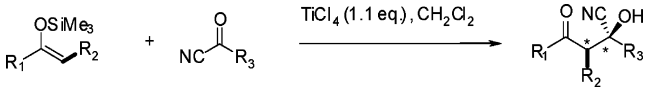


TABLE 1. Synthesis of *β*-Keto-cyanohydrin via a Directed Crossed Aldol between Silyl Enol Ethers and Acyl Cyanides



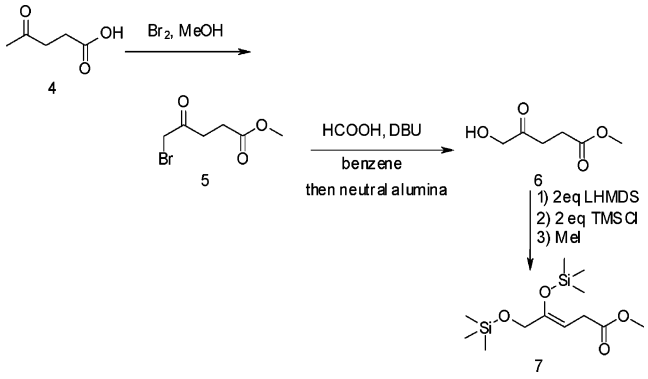
entry	R1	R2	R3	*T* (°C)	*t* (h)	yield (%)
1	CH_3CH_2	CH_3	CH_3	−80	1.5	93
2	C_6H_5	H	CH_3	−80	2	73
3		$-(CH_2)_3-$	CH_3	−80	2	64
4	$PhtCH_2$	H	CH_3	−80	2	58
5	CH_3	$CH_2CO_2CH_3$	CH_3	−80	2	70[a]
6	$PhtCH_2$	$CH_2CO_2CH_3$	CH_3	−20	17	32[a]
7		$-(CH_2)_3-$	C_6H_5	−80	2.5	80
8	CH_3CH_2	CH_3	C_6H_5	−80	2	80
9	C_6H_5	H	C_6H_5	−80	2	95[a]
10	C_6H_5	H	$C(CH_3)_3$	−40	6	15
11	C_6H_5	H	$(CH_2)_2CO_2CH_3$	−80	2	64

[a] Yield of the crude because product hydrolyzes on silica gel.

philic partner had a sufficient reactivity. Under the reaction conditions studied we were not able to react the tetrachlorophthalimido-protected aminoketones.

The reactivity of the silyl enol ethers in the aldol reaction is correlated with the ^{13}C NMR chemical shift of the nucleophilic carbon. The tetrachlorophthalimido-substituted enol ethers should not be reactive in the aldol reaction based on this empirical correlation. Comparing the ^{13}C NMR chemical shifts of the different methyl 5-substituted-4-trimethylsilyloxylevulinates synthesized in our group with the empirical reactivity criteria indicated, the enol ether of methyl 5-silyloxylevulinate **7** should be a valuable candidate for the required aldol coupling. The 5-hydroxy function of compound **7** can be easily transformed into the amino group.

SCHEME 3. Synthesis of the Desired Silyl Enol Ether 7



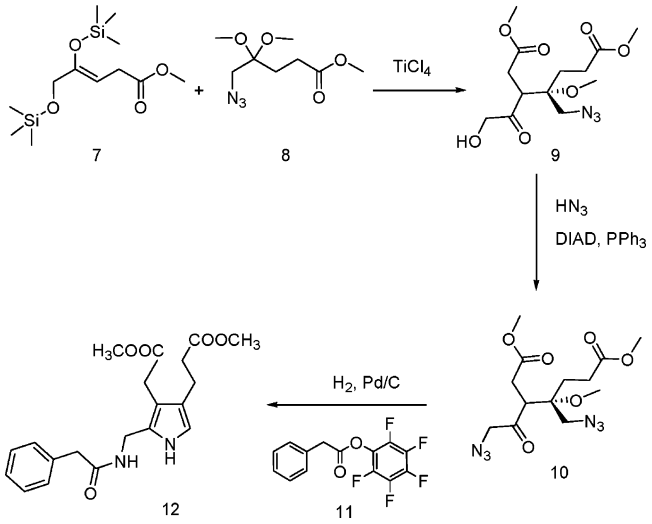
We decided to apply the original strategy developed for the synthesis of alkyl-substituted pyrroles. In these model studies the azido function has been used as a masked equivalent of the amino group present in the electrophilic partner.[10b] The ^{13}C NMR chemical shifts of the silyl enol ethers of methyl 5-azidolevulinate indicate that this compound is not reactive enough for the crossed aldol reaction. The silyl enol ether obtained from 5-protected-hydroxy methyl levulinate fulfils the empirical reactivity criteria and should therefore be a valid reagent for the crossed Mukaiyama aldol reaction. The precursor needed for the synthesis of PBG should be easily accessible. The hydroxyl group can be transformed into the amino group or a precursor of the amine. To obtain the needed silyl enol ether regioselectively we applied the in situ protection procedure of the more acidic methylene group, α to the hydroxy group, by electronic charge repulsion from the alkoxy anion. Treatment of methyl 5-hydroxylevulinate (**6**) with 2 equiv of a strong base and subsequent trapping of the resulting bis-anion by TMS-Cl afforded the desired silyl enol ether **7** in 75% yield after distillation (Scheme 3).

We treated **7** under optimized conditions with the acetal of methyl 5-azidolevulinate (**8**) (Scheme 4). Under these conditions we could isolate 70% of the aldol product **9** as a single diastereoisomer. This intermediate **9** contains the carbon skeleton necessary for the construction of PBG. The relative configuration has not been determined as it is of no consequence for our synthesis endeavor. The introduction of the missing masked amino function was achieved using a Mitsunobu procedure giving **10** in quantitative yield.

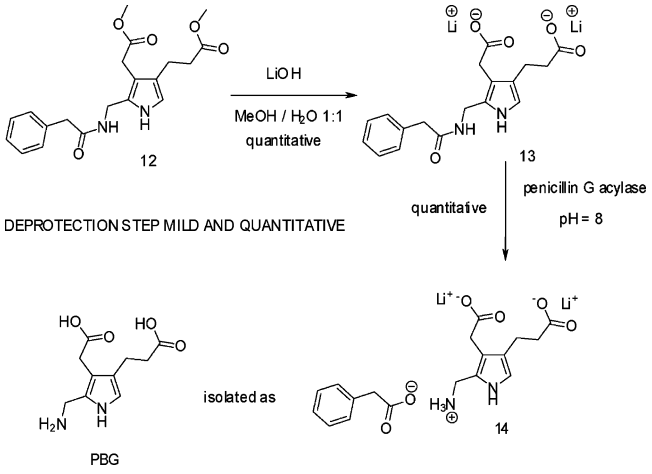
We intended to reduce the more accessible azido function first, followed by the introduction of the phenyl acetyl protecting group. Applying classical Staudinger conditions[17] the desired product could be obtained but only in 32% yield. Applying the Staudinger reaction conditions in the presence of a preformed

(17) Brase, S.; Gil, C.; Knepper, K.; Zimmermann, V. *Angew. Chem., Int. Ed. En.* **2005**, *44*, 5188−240.

SCHEME 4. Synthesis of the Protected Porphobilinogen 12



SCHEME 5. Synthesis of the Phenyl Acetate Ammonium Salt Porphobilinogen 14



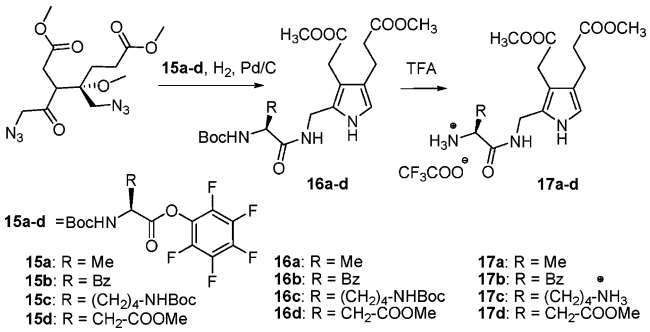
activated ester allowed the isolation of 10% of the corresponding pyrrole **12** (Scheme 4).

This result prompted us to develop a one-pot procedure for the formation of the *N*-phenyl acetyl protected PBG **12** (Scheme 4). Reducing **10** catalytically in the presence of the activated ester **11** allowed the isolation of **12** in 73% yield, as a crystalline solid. We assumed that the more accessible azide was reduced first. Under these conditions the formed amine was rapidly protected as its phenylacetamido derivative. The neopentylic azide was reduced second and the resultant amino ketone spontaneously forms the pyrrole ring.

The ultimate step in our quest for a practical PBG synthesis was the deprotection. Treating the fully protected PBG derivative **12** with 2 equiv of LiOH in a 1:1 mixture of MeOH and water cleaved the two methyl esters (Scheme 5). The saponification was monitored by ^{1}H NMR. After the disappearance of the methyl ester signals, the solvent mixture was adjusted to give an optimal medium for the enzyme penicillin G acylase.[18] After the addition of the enzyme to the aqueous solution containing the dicarboxylate **13**, the pH of the solution was maintained at 8. The enzymatic hydrolysis was complete after 24 h. The

(18) Penicilin G acylase was graciously made available to us by Recordati S. p. A. Milan, Italy.

SCHEME 6. Synthesis *N*-(Amino acid)-Protected Porphobilinogen 17a−d



supported enzyme was removed by filtration and the reaction mixture lyophilized to provide analytically pure PBG, as its phenyl acetate ammonium salt (**14**).

The synthesis sequence reported allows an easy access to PBG derivatives modified at the amino group. PBG derivatives functionalized at the amino group have not been available so far. A potential application of such derivatives is their use as prodrugs for photodynamic therapy (PDT).[19] Our group has shown that bioconjugates between ALA and natural amino acids are interesting prodrugs for the application in PDT.[19c]

We coupled neutral (*Ala* and *Phe*), basic (*Lys*), and acidic (*Asp*) amino acids with the PBG precursor **10** (Scheme 6). The activated esters of the Boc-protected amino acids **15a**−**d** were treated with **10** under reducing conditions described above. The Boc-protected amino acid derivatives of PBG **16a**−**d** were obtained in good yields. Boc deprotection under acidic conditions afforded the amino acid derivatives of PBG **17a**−**d**. The synthetic methodology applied for the synthesis of amino acid derivatives of PBG may be applicable for the synthesis of PBG−protein conjugates as well.[20]

In summary, we have been able to develop a short and efficient synthesis of porphobilinogen, starting from two ALA derivatives. The key step of the synthesis is a Mukaiyama aldol reaction between the regioselectively formed silyl enol ether **7** and the acetal of methyl 5-azidolevulinate **8**. The success of this synthesis relies on the judicious choice of the nucleophilic partner and on its regioselective synthesis. A "one-pot" procedure leads to our designed "ready to use" PBG precursor **12**. The crystalline precursor is stable and free PBG can be released quantitatively under mild conditions. The overall yield of the synthesis is 33% starting from methyl 5-hydroxylevulinate. Efficient synthesis of amino acid derivatives of PBG has been achieved.

Experimental Section

General Experimental Procedures. See the Supporting Information.

Dimethyl 4-Azidomethyl-3-(2-hydroxyacetyl)-4-methoxyheptanedioate (9). Under argon atmosphere, 2.3 g (7.92 mmol, 1.0 equiv) of methyl 4,5-bis(trimethylsilyloxy)pent-3-enoate dissolved in 16 mL of CH_2Cl_2 (treated with basic alox) was cooled to −78 °C then 2.07 g (9.53 mmol, 1.2 equiv) of methyl 5-azido-

(19) (a) Dolmans, D. E. J. G. J.; Fukumura, D.; Jain, R. K. *Nature Rev. Cancer* **2003**, *3*, 380−7. (b) Castano, A. P.; Mroz, P.; Hamblin, M. R. *Nature Rev. Cancer* **2006**, *6*, 535−45. (c) Berger, Y.; Greppi, A.; Siri, O.; Neier, R.; Juillerat-Jeanneret, L. *J. Med. Chem.* **2000**, *43*, 4738−46.

(20) Adamczyk, M.; Fishpaugh, J. R.; Heuser, K. J.; Ramp, J. M.; Reddy, R. E.; Wong, M. *Tetrahedron* **1998**, *54*, 3093−112.

4,4-dimethoxypentanoate[7] in 16 mL of CH_2Cl_2 was added. To the mixture was added 7.6 g (40.2 mmol, 5 equiv) of $TiCl_4$ freshly distilled over polyvinyl pyridine. The mixture was maintained at −55 °C for 16 h, then 75 mL of 2 N NaOH was added and extracted with 5 × 100 mL of $CHCl_3$. The combined organic layer was extracted with 300 mLof satd. NH_4Cl solution, the organic layer was dried over $MgSO_4$, and the solvent was evaporated. The residue was purified by column chromatography (silica gel−100 times the crude mass) using a solvent gradient from 100% hexane to 1:1 hexane:EtOAc. Pure fractions: 1.16 g of dimethyl 4-azidomethyl-3-(2-hydroxy acetyl)-4-methoxyheptanedioate (yield 44.1%). Data for **9**: ^{1}H NMR (400 MHz, $CDCl_3$) 4.47 (dd, ^3J = 5.4 Hz, ^2J = 19.1 Hz, 1H, HC(3^2a)), 4.34 (dd, ^3J = 3.7 Hz, ^2J = 19.1 Hz, 1H, HC(3^2b)), 3.69 (s, 3H, $H_3C(7^1)$), 3.65 (s, 3H, $H_3C(1^1)$), 3.52 and 3.35 (2 × d, AB system, ^2J = 13.3 Hz, 1H each, $H_2C(4^{1\prime})$), 3.31 (dd, $^3J_{3-2a}$ = 2.5 Hz, $^3J_{3-2b}$ = 12.0 Hz, 1H, HC(3)), 3.24 (s, 3H, $H_3C(4^1)$), 2.98 (dd, $^3J_{2a-3}$ = 12.0 Hz, $^2J_{2a-2b}$ = 17.3 Hz, 1H, HC(2a)), 2.45 (dd, $^3J_{2b-3}$ = 2.5 Hz, $^2J_{2b-2a}$ =17.3 Hz, 1H, HC(2b)), 2.37 (ddd, $^2J_{6a-6b} \approx$ 16.0 Hz, $^3J_{6-5b} \approx$ 10.0 Hz, $^3J_{6-5a} \approx$ 6.0 Hz, 2H, HC (6a) and HC(6b)), 2.13 (ddd, $^2J_{5a-5b} \approx$ 15.5 Hz, $^3J_{5a-6b} \approx$ 9.6 Hz, $^3J_{5a-6a} \approx$ 6.0 Hz, 1H, HC (5a)), 1.81 (ddd, $^2J_{5b-5a} \approx$ 15.6 Hz, $^3J_{5b-6a} \approx$ 9.7 Hz, $^3J_{5b-6b} \approx$ 6.0 Hz, 1H, HC (5b)); ^{13}C NMR (100 MHz, $CDCl_3$) 211.9 ($C(3^1)$), 173.6 (C(7), 172.6 (C(1)), 79.0 (C(4)), 71.1 ($C(3^2)$), 54.0 ($C(4^{1\prime})$), 52.6 ($C(1^1)$), 52.4 ($C(7^1)$), 50.7 ($C(4^1)$), 47.7 (C(3)) 32.4 (C(2)) 27.6 (C(6)) 26.1 (C(5)); HR-MS 354.1276 $[M + Na]^+$ (calcd 354.1271).

Dimethyl 3-(2-Azidoacetyl)-4-azidomethyl-4-methoxyheptanedioate (10). Under argon atmosphere 885 mg (3.37 mmol, 1.12 equiv) of **9** was dissolved in 35 mL of benzene and the solution was cooled to 5−10 °C, then 680 mg (3.36 mmol, 1.11 equiv) of DIAD followed by 1000 mg (3.02 mmol, 1.0 equiv) of dimethyl 4-azidomethyl-3-(2-hydroxyacetyl)-4-methoxyheptanedioate dissolved in 15 mL benzene were added dropwise. Finally 3 mL (0.2 g, 6.88% w/v, 4.8 mmol, 1.6 equiv) of a HN_3 solution in benzene (HN_3 generated by adding 1 mL of 98% H_2SO_4 to 2.27 g of NaN_3 in 3 mL of water) was added dropwise. The mixture was maintained at 10 °C for 2−3 h. The solvent was evaporated. The residue was purified by column chromatography on silica gel with a CH_2Cl_2: EtOAc solvent gradient from 95:5 to 80:20, then 1.0 g of pure dimethyl 3-(2-azidoacetyl)-4-azidomethyl−4-methoxyheptanedioate was obtained (yield 93.0%). Data for **10**: ^{1}H NMR (400 MHz, $CDCl_3$) 4.22 (d, ^2J = 18.3 Hz, 1H, HC(3^2a)), 4.14 (d, ^2J = 18.3 Hz, 1H, HC(3^2b)), 3.68 (s, 3H, $H_3C(7^1)$), 3.65 (s, 3H, $H_3C(1^1)$), 3.55 and 3.36 (2 × d, AB system, ^2J = 13.2 Hz, 1H each, $H_2C(4^{1\prime})$), 3.24 (dd, $^3J_{3-2a}$ = 2.5 Hz, $^3J_{3-2b}$ = 12.0 Hz, 1H, HC(3)), 3.23 (s, 3H, $H_3C(4^1)$), 2.98 (dd, $^3J_{2a-3}$ =12.2 Hz, $^2J_{2a-2b}$ =19.0 Hz, 1H, HC(2a)), 2.42 (dd, $^3J_{2b-3}$ =2.5 Hz, $^2J_{2b-2a}$ =17.3 Hz, 1H, HC-(2b)), 2.50−2.23 (m, 2H, $H_2C(6)$), 2.14 (ddd, $^2J_{5a-5b} \approx$ 15.5 Hz, $^3J_{5a-6b} \approx$ 9.5 Hz, $^3J_{5a-6a} \approx$ 6.0 Hz, 1H, HC (5a)), 1.79 (ddd, $^2J_{5b-5a} \approx$15. 6 Hz, $^3J_{5b-6a} \approx$ 9.7 Hz, $^3J_{5b-6b} \approx$ 6.0 Hz, 1H, HC (5b)); ^{13}C NMR (100 MHz, $CDCl_3$) 206.2 ($C(3^1)$), 173.2 (C(7)), 172.4 (C(1)), 79.0 (C(4)), 60.2 ($C(3^2)$), 53.4 ($C(4^{1\prime})$), 52.3 ($C(1^1)$), 52.1 ($C(7^1)$), 50.4 ($C(4^1)$), 48.7 (C(3)), 32.4 (C(2)), 27.3 (C(6)), 25.6 (C(5)); HR-MS 379.13371 $[M + Na]^+$ (calcd 379.13365).

Methyl 3-[4-Methoxycarbonylmethyl-5-(phenylacetylaminomethyl)-1*H*-pyrrol-3-yl]propionate (11). A suspension of 62 mg of Pd/C in 10 mL of MeOH was pre-hydrogenated at ambient temperature for 15 min, then 1.208 g (4 mmol, 2 equiv) of pentafluorophenylphenyl acetate (**6**) was added followed by a solution of 712 mg (2 mmol) of dimethyl *rac*-3-(2-azidoacetyl)-4-azidomethyl-4-methoxyheptanedioate in 25 mL of MeOH. The reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 14 h. The mixture was then filtered over celite and the solvent was evaporated. The residue obtained was purified by flash chromatography with the solvent mixture *n*-hexane/EtOAc (35:65) and 500 mg (72%) of methyl 3-[4-methoxycarbonylmethyl-5-(phenylacetylaminomethyl)-1*H*-pyrrol-3-yl]propionate (**11**) was obtained as an oily substance. Data for **11**: ^{1}H NMR (400 MHz, $CDCl_3$) 9.00 (s large, 1H, NH-pyrrole), 7.40−7.25 (m, 5H, H aromatic), 6.54 (t large, 1H, NH-amide), 6.44 (d, ^3J(2,NH) = 2.6 Hz, 1H, H−C(2)), 4.28 (d, $^3J(5^1$,NH) = 5.8 Hz, 2H, $H_2-C(5^1)$), 3.68 (s, 3H, $H_3-C(3^4)$), 3.65 (s, 3H, $H_3-C(4^3)$), 3.54 (s, 2H, $H_2-C(5^3)$), 3.44 (s, 2H, $H_2-C(4^1)$), 2.73 (tripletoïde, $^3J(3^1,3^2) \approx$ 7.7 Hz, $H_2-C(3^1)$), 2.55 (tripletoïde, $^3J(3^2,3^1) \approx$ 7.9 Hz, $H_2-C(3^2)$); ^{13}C NMR (100 MHz, $CDCl_3$) 173.9 ($C(3^3)$), 173.4 ($C(4^2)$), 172.1 ($C(5^2)$), 135.5 ($C(5^4)$), 129.7 ($C(5,^5\ 5^{5\prime})$), 129.0 ($C(5,^6\ 5^{6\prime})$), 127.7 (C(5)), 127.4 ($C(5^7)$), 121.5 (C(3)), 114.6 (C(2)), 111.9 (C(4)), 52.2 ($C(4^3)$), 51.7 ($C(3^4)$), 43.7 ($C(5^3)$), 35.3 ($C(5^1)$), 35.1 ($C(3^2)$), 30.0 ($C(4^1)$), 20.8 ($C(3^1)$). Anal. Calcd for $C_{20}H_{24}N_2O_5 + 0.16H_2O$: C, 64.00; H, 6.48; N, 7.46. Found: C, 63.95; H, 6.55; N, 7.23.

4-(2-Carboxyethyl)-3-carboxymethyl-1*H*-pyrrol-2-ylmethylammonium Phenyl Acetate (12). To a solution of 372 mg (1 mmol, 1 equiv) of methyl 3-[4-methoxycarbonylmethyl-5-(phenylacetylaminomethyl)-1*H*-pyrrol-3-yl]propionate (**7**) in 15 mL of a MeOH/H_2O (1:1) mixture was added 84 mg (2 mmol, 2 equiv) of lithium hydroxide monohydrate. The solution was stirred at room temperature. The saponification was complete after 20 h (^{1}H NMR).

To this solution was added 18 mL of H_2O and the pH was adjusted to 8 using diluted HCl. A suspension of 420 mg (88 IU) of penicillin G acylase in 25 mL of H_2O was added. The amide hydrolysis was complete after 20 h. The enzyme was removed by filtration and the filtrate was lyophilized to give the hydrolyzed product 4-(2-carboxyethyl)-3-carboxymethyl-1*H*-pyrrol-2-ylmethylammonium phenyl acetate (**12**). Data for **12**: ^{1}H NMR (400 MHz, CD_3OD) 7.33−7.22 (m, 4H, H aromatic $PhAcO^-$), 7.19−7.15 (m, 1H, H aromatic $PhAcO^-$), 6.53 (s, 1H, H−C(5)), 3.99 (s, 2H, $H_2-C(2^1)$), 3.49 (s, 2H, H_2C $PhAcO^-$), 3.37 (s, 2H, $H_2-C(3^1)$), 2.75 (tripletoïde, $^3J(4^1,4^2) \approx$ 7.8 Hz, $H_2-C(4^1)$), 2.40 (tripletoïde, $^3J(4^2,4^1) \approx$ 7.9 Hz, $H_2-C(4^2)$); ^{13}C NMR (100 MHz, CD_3OD) 181.6 ($C(4^3)$), 180.1 ($C(3^2)$), 179.4 (C═O $PhAcO^-$), 138.3 (C quaternary arom. $PhAcO^-$), 129.2, 128.1, 125.9 (C aromatic $PhAcO^-$), 123.3 (C(4)), 120.8 (C(2)), 118.1 (C(3)), 115.3 (C(5)), 45.4 (CH_2 $PhAcO^-$), 39.1($C(4^2)$), 34.8 ($C(2^1)$), 33.8 ($C(3^1)$), 22.3 ($C(4^1)$); ESI-MS $[M]^-$ 225.2.

Acknowledgment. This research was supported by the Swiss National Science Foundation and the University of Neuchatel. NMR and mass spectra were measured on instruments at the Universities of Neuchatel and Fribourg acquired with the assistance of grants from the Swiss National Science Foundation.

Supporting Information Available: Full experimental procedures and characterization data for all new products. This material is available free of charge via the Internet at http://pubs.acs.org.

JO702319N