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New Hydrophobic Vitamin B_{12} Derivatives via Ring-Opening Reactions of *c*-Lactone

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ABSTRACT

A selective synthesis of new hydrophobic cobalamin derivatives bearing two different spacers has been accomplished via ring-opening reaction of *c*-lactone. The reaction of *c*-lactone with various amines afforded three types of amides (a, b, and c) depending on the reaction conditions. The structure of lactone b was determined by the X-ray analysis confirming the position of ring closure. It also reveals the presence of a hydrogen bond between the terminal hydroxy group and one of the axial cyanide ligands.

Independent of its importance as an essential nutrient cofactor for all mammals, vitamin B_{12} has received a lot of attention over the past decade.[1] This is mainly due to its use in the targeted delivery of therapeutic agents.[2] Vitamin B_{12} is a highly functionalized molecule that can be modified for conjugation with other compounds. It can be derivatized at the Co metal center via alkylation[3] or through functionalization of the 5′-hydroxyl group on the ribose moiety.[4] Coupling through the carboxylates sites produced from acidic hydrolysis of the propionamide side chains is also common.[5,6]

Hydrophobic cobalamin derivatives have been used as model compounds and as artificial enzymes in many reactions.[7] In particular, heptamethyl cobyrinate perchlorate [Co(II)7C_1ester]ClO_4 was found to catalyze dehalogenation of chlorinated organic compounds.[8] The most widely studied hydrophobic derivatives are compounds having ester groups in place of the peripheral amide moieties of natural vitamin B_{12}.[9,10] Hisaeda and co-workers used these compounds to

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(1) Brown, K. L. *Chem. Rev.* **2005**, *105*, 2075–2150.
(2) (a) Petrus, A. K.; Fairchild, T. J.; Doyle, R. P. *Angew. Chem., Int. Ed.* **2009**, *48*, 1022–1028. (b) Fedosov, S. N.; Fedosova, N. U.; Kräutler, B.; Nexø, E.; Petersen, T. E. *Biochemistry* **2007**, *46*, 6446–6458. (c) Wuerges, J.; Garau, G.; Geremia, S.; Fedosov, S. N.; Pertersen, T. E.; Randaccio, L. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 4386–4391. (d) Chalasani, K. B.; Russell-Jones, G. J.; Jain, A. K.; Diwan, P. V.; Jain, S. K. *J. Controlled Release* **2007**, *122*, 141–150. (e) Bagnato, J. D.; Eilers, A. L.; Horton, R. A.; Grissom, Ch. B. *J. Org. Chem.* **2004**, *69*, 8987–8996. (f) Russell-Jones, G. J.; McEwan, J. F. U.S. Patent US006150341A, 2000.
(3) (a) Shimakoshi, H.; Maeyama, Y.; Kaieda, T.; Matsuo, T.; Matsui, E.; Naruta, Y.; Hisaeda, Y. *Bull. Chem. Soc. Jpn.* **2005**, *78*, 859–863. (b) Mokherjee, R.; Donnay, E. G.; Radomski, M. A.; Miller, C.; Redfern, D. A.; Gericke, A.; Brasch, N. E. *Chem. Commun.* **2008**, 3783–3785. (c) Murakami, Y.; Hisaeda, Y.; Kohno, H.; Ohno, T. *Chem. Lett.* **1992**, 909–912. (d) Hisaeda, Y.; Nishioka, T.; Inoue, Y.; Asada, K.; Hayashi, T. *Coord. Chem. Rev.* **2000**, *198*, 21–37.
(4) (a) Wang, X.; Wei, L.; Kotra, L. P. *Bioorg. Med. Chem.* **2007**, *15*, 1780–1787. (b) McEwan, J. F.; Veitch, H. S.; Russell-Jones, G. J. *Bioconjugate Chem.* **1999**, *10*, 1131–1136.
(5) (a) Otten, T.; Dabre, T.; Cosnier, S.; Lusia, A.; Correia, J.; Keese, R. *Helv. Chim. Acta* **1998**, *81*, 1117–1126. (b) Russell-Jones, G.; Westwood, S.; Farnworth, P.; Findlay, J.; Burger, H. *Bioconjugate Chem.* **1995**, *6*, 34–42.
(6) Pathare, P. M.; Wilbur, D. S.; Hausser, S.; Quadros, E. V.; McLoughlin, P.; Morgan, A. Ch. *Bioconjugate Chem.* **1996**, *7*, 217–232.
(7) Ohno, T.; Ogawa, A.; Hisaeda, Y.; Murakami, Y. *J. Chem. Soc., Perkin Trans. 2* **1994**, *2*, 2271–2273.
(8) Jabbar, Md. A.; Shimakoshi, H.; Hisaeda, Y. *Chem. Commun.* **2007**, 1653–1655.

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prepare organic/inorganic hybrid molecules that act as artificial enzymes.[11]

Another important hydrophobic derivative is the hexaester-*c*-monoacid. This useful synthetic precursor is often obtained via selective lactonization, followed by in situ reduction with zinc.[12] It has also been prepared via hydrolysis followed by esterification; however, a HPLC separation is required.[13] This compound may be coupled readily with amines or alcohols.[5,12]

While investigating vitamin B_{12}-biotin conjugates Wilbur et al. carried out the ring-opening reaction of a hydrophilic *c*-lactone in melt diaminododecane.[6] A similar approach was described by Alberto et al., using 1,4-diaminobutane as a nucleophile.[14] To the best of our knowledge there are no reports on such ring-opening reactions involving hydrophobic *c*-lactone **1**.

Preliminary results showed that the ring-opening reaction of *c*-lactone **1** with amines in dioxane led to a mixture of mono- and diamides depending on the nucleophile. Since the selective introduction of functionalities into vitamin B_{12} and its derivatives has long been a challenge we decided to study this reaction in depth with the goal of finding specific reaction conditions for mono- or diamide formation.

Our investigation began by carrying out a series of experiments involving ethanolamine (Scheme 1). The reaction of *c*-lactone **1a** with 10 equiv of this amine in dioxane gave exclusively monoamide **2** in 72% yield (Table 1, entry 1). When the reaction was carried out for a longer period of time or conducted in DCM the yield was reduced and selectivity was lost (entries 2 and 3). Using 4 equiv of amine assured selective formation of **2** in dioxane and DCM, although in the latter case the formation of a new compound was observed (entry 5, spirolactone). By increasing the temperature, the reaction could be completed faster; however, this also promoted the formation of unwanted byproducts (entries 6 and 7).

Reactions carried out in DCM proved more favorable for diamide **3** formation; consequently, this solvent was used for the optimization studies (Table 2). By increasing the amine concentration, diamide **3** was obtained as a major product; however, the reaction was not fully selective (entries 1−3). The best result, 75% yield of **3**, was obtained when the reaction was stopped after 6 h (entry 2). When lactone **1b** was used as the starting material (entries 4 and 6),[15] the reaction kinetics were improved, but more byproducts were formed (entry 4).

An unexpected result was found when an amine/solvent ratio of 1:1 was used. While the yield of the diamide **3** decreased dramatically, neither monoamide **2** nor the starting material **1** was detected (entries 5 and 6).

All reactions subject to study proved concentration and solvent dependent; therefore, a series of solvents with

Scheme 1. Reaction of Lactone **1** with Ethanolamine

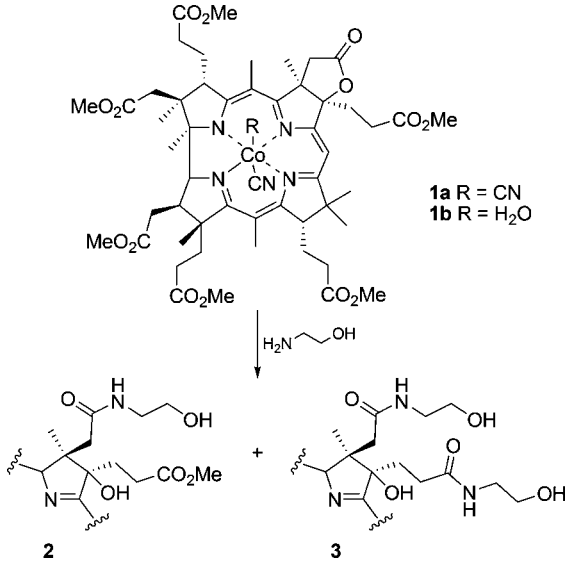
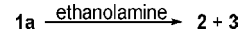


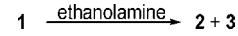
Table 1. Ring-Opening of Lactone **1a** by Ethanolamine[a]



entry	amine [equiv]	solvent	*t* [°C]	time [h]	amide **2** [%][b]	diamide **3** [%][b]
1	10	dioxane	rt	5	72	
2	10	dioxane	rt	16	68	20
3	10	DCM	rt	5	56	18
4	4	dioxane	rt	29	72	
5[c]	4	DCM	rt	29	61	
6	4	dioxane	50	16	38	37
7	4	DCM	50	6	57	

[a] All reactions were protected from light. [b] Isolated yields. [c] Spirolactone **4** was formed in 11% yield.

Table 2. Diamide **3** Formation via the Reaction of *c*-Lactone **1** with Ethanolamine[a]



entry	lactone	amine/solvent ratio	time [h]	amide **2** [%][b]	diamide **3** [%][b]
1	**1a**	10:1	3	10	43
2	**1a**	10:1	6	19	75
3	**1a**	10:1	16	trace	57
4	**1b**	10:1	0.7	48	32
5	**1a**	1:1	3		37
6	**1b**	1:1	0.7		29

[a] Reactions were carried out at room temperature in DCM. [b] Isolated yields.

(9) Müller, S.; Wolleb, A.; Walder, L.; Keese, R. *Helv. Chim. Acta* **1990**, *73*, 1659–1669.

(10) Murakami, Y.; Hisaeda, Y.; Ohno, T. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 2091–2097.

(11) Hisaeda, Y.; Masuko, T.; Hanashima, E.; Hayashi, T. *Sci. Technol. Adv. Mater.* **2006**, *7*, 6555–661.

(12) Pfammatter, M. J.; Dabre, T.; Keese, R. *Helv. Chim. Acta* **1998**, *81*, 1105–1106.

different polarities were tested. As the polarity of the solvent decreased, the reaction rate increased (see SI).

Using an optimal set of conditions, a small collection of amines was examined (Table 3).[16] In the case of simple aliphatic amines (*n*-butyl amine, isopropylamine, and *tert*-butylamine), an effect ascribable to sterics was observed: as the bulk bound to the amine increased the reactivity decreased. Monoamides from both *n*-butyl- and *n*-octylamine were obtained in high yield. For propargylamine the low yield was improved by increasing the amine concentration. Unfortunately, in this specific case we were not able to obtain diamide.

Table 3. Scope and Limitations Studies

entry	amine	monoamide [%][a]	diamide [%][b]
1	ethanolamine	72	75
2	diglycolamine	71	70
3	*n*-butylamine	65	53
4	*n*-octylamine	67	40
5	isopropylamine	25	58
6	*tert*-butylamine	0	0
7	benzylamine	0	0
8	propargylamine	10	
9	propargylamine	68[c]	

[a] Conditions: lactone 0.14 mM in dioxane with 10 equiv of amine. [b] Conditions: lactone 0.14 mM in DCM/amine mixture (10:1), rt. [c] Conditions: lactone 0.14 mM in dioxane/amine (10:1), rt.

In the reaction of lactone **1a** with 10 equiv of ethanolamine in DCM, the formation of a new compound **4** was observed. Single crystals suitable for X-ray diffraction analysis were obtained by difusion of Et_2O into an ethanol solution of **4** (Figure 1). The resulting structure revealed that the spirolactone ring is formed at the corrin B-ring. The terminal hydroxy group at the neighboring position forms an intramolecular hydrogen bond with the cyanide anion. We assume that the formation of a hydrogen bond between −OH and −CN stabilizes the amides of this report based on the fact that ^{1}H NMR spectra are simpler than those obtained for compounds without a terminal hydroxy group. In these instances, almost all signals are split with the second set of signals being atributed to the species devoid of one cyanide ligand, as proposed by Alberto.[14] The general features of corrin geometry are similar to those seen earlier.[17]

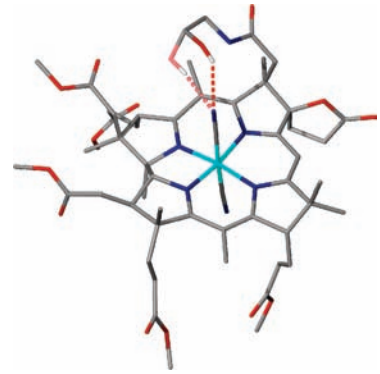


Figure 1. X-ray structure of spirolactone **4**.

The coordination sphere around the central Co atom is a distorted octahedron formed by four N atoms coming from the corrin ring and two axial C atoms from cyanide anions. The distances to N atoms from rings A and D are slightly shorter than those to N atoms from C and rings (Co−N 1.867(4) Å, Co−N 1.905(4) Å, Co−N 1.919(4) Å, Co−N 1.870(4) Å). The arrangement of the corrin ring around the Co center is tetrahedrally distorted with a folding angle of 10.8° (angle between the plane passing through the A and B rings and the plane passing through the C and D rings). The folding angle is slightly smaller than typically observed for cobalamines. This can be attributed to the presence of less bulky axial ligand (cyanide vs substituted imidazole).

We found that spirolactone **4** is an intermediate formed during the synthesis of the diamide **3** and is sometimes a byproduct in the synthesis of the monoamide **2**. It is known that cyanides are efficient and mild catalysts in the aminolysis of esters[18] and this ligand is always present in the reaction mixtures as the result of starting with a cobalamine derivative. Furthermore, it has been briefly mentioned by Gossauer that *d*-lactonization can occur as a result of a cyanide group being present.[19] Therefore, we assumed that the monoamide **2** forms first, followed by *d*-lactonization to give **4**. A subsequent ring-opening with a second molecule of amine then gives **3** (Table 4). It was thus thought that increasing the concentration of cyanide ions would abet the formation of spirolactone **4**.

(13) Wilson, S.; Reinberd, K. S.; Gao, X. U.S. Patent US 2007/0066561 A1, 2007.

(14) Staveren, D. R.; Waibel, B.; Mundwiler, S.; Schubiger, P. A.; Alberto, R. *J. Organomet. Chem.* **2004**, *689*, 4803–4810.

(15) Murakami, Y.; Hisaeda, Y.; Ozaki, T. *Chem. Lett.* **1985**, 473–476.

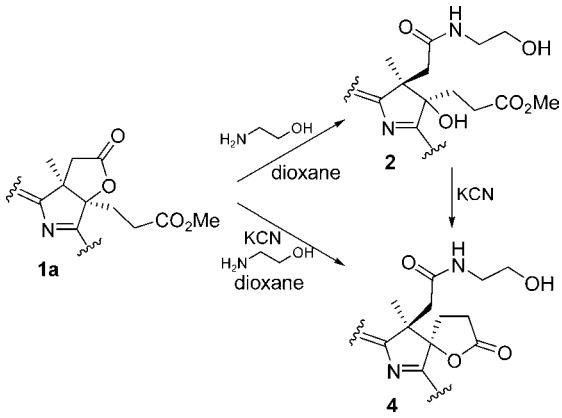
(16) **Synthesis of mono-amides:** Lactone (0.014 mmol) and the primary amine (0.14 mmol) were dissolved in dioxane (1 mL). The mixture was stirred at room temperature, under an argon atmosphere, for the specified time. It was then diluted with DCM, washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was then purified by DCVC, using the specified concentration of ethanol in DCM. The isolated pure product was then redissolved in DCM, treated with KCN, and allowed to stand for 5−10 min. The KCN was removed by filtration and the filtrate concentrated in vacuo. The mono-amide was then recrsytallized with ethyl acetate and hexane and dried in low vacuum, producing a purple crystalline product. **Synthesis of diamides:** Lactone (0.014 mmol) was dissolved in DCM (1 mL) followed by the addition of the primary amine (0.1 mL). The mixture was stirred at room temperature, under an argon atmosphere, for the specified time. The remaining workup follows the procedure of mono-amides.

(17) (a) Randaccio, L.; Furlan, M.; Geremia, S.; Šlouf, M.; Srnova, I.; Toffoli, D. *Inorg, Chem.* **2000**, *39*, 3403–3413. (b) Randaccio, L.; Geremia, S.; Nardin, G.; Slouf, M.; Srnova, I. *Inorg. Chem.* **1999**, *38*, 4087–4092. (c) Randaccio, L.; Furlan, M.; Geremia, S.; Slouf, M. *Inorg. Chem.* **1998**, *37*, 5390–5393. (d) Randaccio, L.; Geremia, S.; Stener, M.; Toffoli, D.; Zangrando, E. *Eur. J. Inorg. Chem.* **2002**, 93–103.

(18) (a) Högberg, T.; Ström, R.; Ebner, M.; Rämsby, S. *J. Org. Chem.* **1987**, *52*, 2033–2036. (b) Basabe, P.; Blanco, A.; Bodero, O.; Martín, M.; Marcos, I. S.; Díez, D.; Mollinedo, F.; Urones, J. G. *Tetrahedron* **2010**, *66*, 2422–2426. (c) Reyes-Gutiérrez, P. E.; Torres-Ochoa, R. O.; Martínez, R.; Miranda, L. D. *Org. Biomol. Chem.* **2009**, *7*, 1388–1396. (d) Fürstner, A.; Guth, O.; Rumbo, A.; Seidel, G. *J. Am. Chem. Soc.* **1999**, *121*, 11108–11113.

(19) Grüning, B.; Holze, G.; Jenny, T. A.; Nesvadba, P.; Gossauer, A. *Helv. Chim. Acta* **1985**, *68*, 1745–1769.

Table 4. Spirolactone **4** Formation[a]



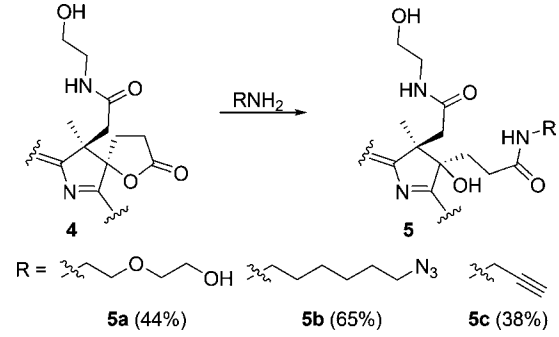
entry	KCN [amount]	time [h]	amide **2** [%][b]	spirolactone **4** [%][b]
1[c]	catalytic	48	59	trace
2[c]	stiochiometric	48		29
3[c]	excess	48		45
4[d]	catalytic	48		
5[d]	stiochiometric	48		37
6[d]	excess	48		83
7[e]	excess	24		81

[a] All reactions were carried out with 4 equiv of ethanolamine in dioxane at rt. [b] Isolated yields. [c] Lactone **1** was used as substrate (one step procedure). [d] Amide **2** was used as a substrate.

A catalytic amount of KCN was added to the reaction mixture and only the yield of monoamide **2** decreased (Table 4, entry 1). The use of a large excess of the KCN led to selective formation of spirolactone **4**, albeit only in 45% yield (entry 3). The low yields could reflect the fact that KCN affects the formation of amide **2**, which once formed has been shown to give **4** under these conditions. In fact, by isolating **2** first, and then treating with cyanide, the spirolactone **4** could be isolated in 83% yield (entry 6). Unfortunately, reaction times of ca. 48 h were required. These long reaction times could reflect the low solubility of KCN in dioxane.[20] Therefore, TBACN was investigated as an alternative cyanide source. In this case, excelent yields were obtained and in a shorter period of time; however, a minor byproduct is formed, which renders purifcation more difficult (entry 7).

Spirolactone **4** provides a new reaction site that allows the formation of diamides with two different substituents starting with primary amines that possess terminal groups, such as NH_2, N_3, and alkyne. As shown in Scheme 2, derivatives **5a**−**c** could be obtained in this way.

Scheme 2. Selective Ring-Opening of Spirolactone **4**



Lactone **4** was treated with 10 equiv of appropriate amine in dioxane.

In summary, we have developed a selective ring-opening of vitamin B_{12} derived *c*-lactone **1** that produces *c*-mono-**2** or *c*,*d*-diamides, such as **3** and **5**. The optimal conditions for the production of monoamide **2** involved using 10 equiv of an amine in dioxane at room temperature. In contrast, the use of a 10:1 ratio in DCM at room temperature gave diamide **3**. The aminolysis of **2** in the presence of KCN provided *d*-spirolactone **4** in good yield. Subsequent ring-opening gave *c*,*d*-diamides **3**. This operationally simple method provides access to cobalamin-derived diamides of general structure **5** that possess various substituents at the *c*- and *d*-positions.

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Supporting Information Available: Experimental details and complete analytical data of all new products. This material is available free of charge via the Internet at http://pubs.acs.org. The crystallographic coordinates have been deposited with the Cambridge Crystallographic Data Centre; deposition no. 789895. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre, 12 Union Rd., Cambridge CB2 1EZ, UK or via www.ccdc.cam.ac.uk/conts/retrieving.html.

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(20) Rzasa, R. M.; Shea, H. A.; Romo, D. *J. Am. Chem. Soc.* **1998**, *120*, 591–592.