Efficient Synthesis of ^{14}C-Labeled 1*H*-Pyrazolo[3,4-*d*]pyrimidine and Related [4.3.0]-Bicyclic Pyrimidino Systems

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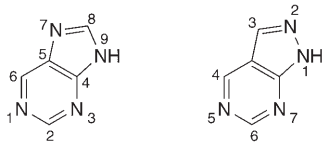
J. Z. H. dedicates this paper to his postdoctoral research advisor, the late Professor *Henry Rapoport*, Ph.D., Department of Chemistry, University of California, Berkeley, California 94720, USA, on the occasion of his 90th birthday.

In support of a research program aimed at discovering purine-related anticancer drug candidates, a method for the ^{14}C-labeling of pyrazolopyrimidines utilizing the readily available labeled starting material, sodium [^{14}C]formate, has been developed with a good overall yield. This new method was proven to be general in the preparation of other related [4.3.0]heterocycles containing N, O, and S atoms. A concise synthesis of a model compound, 8-aza-7-deaza-5′-[^{14}C]noraristeromycin, was achieved utilizing this methodology as a key step.

Introduction. – 9*H*-Purine (**1**) and 1*H*-pyrazolo[3,4-*d*]pyrimidine (**2**) are a class of molecules that have a five-membered ring fused to a six-membered ring. In each ring, there are two N-atoms, but there is no N-atom at the ring junction (*Figure*)[1]). The biological activity of compounds that contain these ring systems has generated increased interest in their chemistry [2]. It was reported that many purine nucleosides and nucleotides are anticancer or antiviral agents [3]. 1*H*-Pyrazolo[3,4-*d*]pyrimidine (**2**), an isomer of purine, was widely used as a purine replacement in the synthesis of compounds aimed at improved target activity [4]. An efficient synthetic route to prepare ^{14}C-labeled 4-hydroxy-1*H*-pyrazolo[3,4-*d*]pyrimidine is important to build radiolabeled tracers used in absorption, distribution, metabolism, and excretion (ADME) studies, and investigations of activation potentials [5].



1 **2**

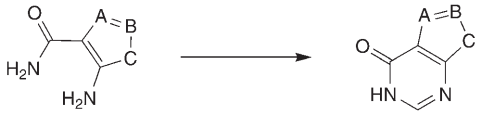
Figure. *Structures of 9*H*-purine* (**1**) *and 1*H*-pyrazolo[3,4-*d*]pyrimidine* (**2**)

[1]) For recent reviews on purine and related compounds, see [1a][1b]. For reviews on 1*H*-pyrazolo[3,4-*d*]pyrimidine and related compounds, see [1c].

Results and Discussion. – The preparation of 1,5-dihydro-4*H*-pyrazolo[3,4-*d*]-pyrimidin-4-one (**4**) from cyclocondensation of 5-amino-1*H*-pyrazole-4-carboxamide (**3**) with triethyl orthoformate (*Table*, *Entry 1*) was previously documented in the literature, but the yields of the reaction were generally low [6]. Although better yields can be achieved by replacement of triethyl orthoformate with formamide (*Table*, *Entry 2*) [7], ^{14}C-labeled formamide is expensive and not readily available. Given the frequent appearance of building block **2** in pharmaceutical compounds, we sought to expand the scope of this potentially useful cyclization method and optimize the efficiency. In the present work, a simple procedure by which sodium formate (readily available in ^{14}C-labeled form) was utilized to construct **4** and its analogs was established.

We have found that heating a mixture of sodium formate and a β-aminocarboxamide as intimately mixed solids can directly afford the desired purine or pyrazolopyrimidine. A range of related [4.3.0]heterocycles were explored and found to be obtainable by this new method (*Table*, *Entries 3–7*). The reactions were conducted in a solvent-free manner with three equivalents of sodium formate, and the yields were moderate to good (61–89%), based on the utilized β-aminocarboxamide. This method is generally applicable, because it tolerates a variety of heteroatoms such as N, O, and S.

Table. *Formation of Unlabeled [4.3.0]Pyrimidino Heterocycles* via *Cyclocondensation of β-Aminocarboxamides*



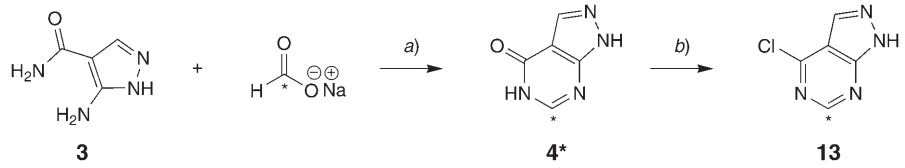
Entry	*β*-Aminocarboxamide				Condition	Solvent	Time [h]	[4.3.0]-Heterocycle	Yield [%][a])
	Compound	A	B	C					
1	**3**	H–C	N	H–N	$HC(OEt)_3$, 80°	Ac_2O	4	**4**	11 [6]
2	**3**	H–C	N	H–N	formamide, 190°	formamide	1	**4**	85 [7]
3	**3**	H–C	N	H–N	HCO_2Na, 190°	none	4	**4**	89
4	**5**	N	H–C	H–N	HCO_2Na, 190°	none	4	**6**	77 [8]
5	**7**	Me–C	N	O	HCO_2Na, 190°	none	4	**8**	61 [9]
6	**9**	H–C	N	Ph–N	HCO_2Na, 190°	none	4	**10**	81 [10]
7	**11**	Me–C	Ph–C	S	HCO_2Na, 190°	none	4	**12**	62

[a]) All yields refer to isolated products.

The ^{14}C-labeled 1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one, **4***, was prepared by cyclocondensation of 5-amino-1*H*-pyrazole-4-carboxamide (**3**) with ^{14}C-labeled sodium formate (*Scheme 1*). When one equivalent of sodium [^{14}C]formate was used, only 35% of **3** were converted to **4***, as indicated by HPLC. Separation of **4*** from **3** was very difficult. Alternatively, heating of **3** with three equivalents of [^{14}C]HCO_2Na in a sealed tube at 190° for 4 h resulted in a 90% conversion of **3**. After FC, the desired product **4*** was obtained in 69% yield, based on **3**. In radiochemistry, scientists often report radiochemical yields, which is calculated based on the consumption of the

radiochemical starting material (SM) regardless whether it is the limiting reagent or not. In our case, the radiochemical SM was [^{14}C]HCO_2Na, and it was used in threefold excess with respect to the limiting reagent **3**. Therefore, the radiochemical yield of this reaction, calculated on the basis of the consumption of [^{14}C]HCO_2Na, was 23% (69% × 1/3 = 23%).

Scheme 1

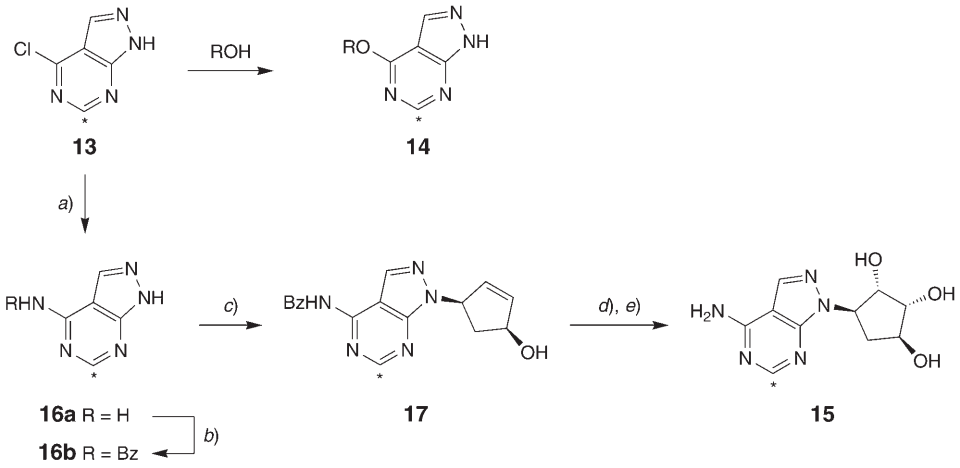


a) 190°, 4 h, 23%. *b*) $POCl_3$, CH_2Cl_2, r.t., 16 h, 88%.

Chlorination of **4*** generates the chloride **13** (*Scheme 1*), a very reactive intermediate. Reaction of chloride **13** with an alcohol, either aliphatic or aromatic, under reflux in $CHCl_3$, would give rise to a pyrimidine ether **14** (*Scheme 2*). Since a large number of alcohols are available either commercially or synthetically, this method should easily lead to many ^{14}C-labeled pyrimidine (or purine) tracers for ADME and other metabolism related studies.

Furthermore, the Cl group in **13** can be transformed into an amino group, and that would be a crucial step in the synthesis of many nucleoside tracers. Therefore, we decided to synthesize ^{14}C-labeled 8-aza-7-deaza-5′-noraristeromycin (**15**), a natural

Scheme 2



a) NH_4OH, reflux 2 h; 71%. *b*) BzCl, pyridine, r.t., 4 h; 94%. *c*) (1*R*,4*S*)-4-Hydroxycyclopent-2-en-1-yl acetate, $NaN(TMS)_2$, $(Ph_3P)_4Pd$, Ph_3P, DMSO, THF, 60°; 44%. *d*) OsO_4, NMO, THF, H_2O. *e*) NH_3/MeOH; 51%.

product, to demonstrate the synthetic potential of this newly developed labeling technique. Treatment of ^{14}C-labeled **13** with NH_4OH in THF at 85° yielded amino compound **16a**, which was converted into benzamide **16b** by benzoylation. The latter was successfully transformed to the desired tracer **15** in three steps, in analogy to a published procedure [4a] (*Scheme 2*).

In conclusion, a novel method for the preparation of ^{14}C-labeled pyrazolopyrimidines from the readily available inexpensive reagent sodium [^{14}C]formate was developed. It was demonstrated that this procedure is general and can be used for the synthesis of other related [4.3.0]heterocycles.

Experimental Part

General. Anh. solvents were obtained from *Aldrich* and were dried over 4-Å molecular sieves for at least 24 h prior to use. All of the commercially available reagents and solvents were from either *Aldrich Chemical Co.*, USA, or from *Fisher Scientific Co.*, USA, except compounds **5**, **7**, **9**, and **11**; they were purchased from *Trega Biosciences, Inc.*, 16140 El Camino Real, Rancho Santa Fe, CA 92067, USA. Analytical HPLC: *Shimadzu* HPLC System with *LC-10ATVP* pumps, *SPD-10AVP* UV detector, *CTO-10ASVP* column oven heated to 30°, a *SCL-10A* controller and a *Packard Radiomatic*™ *150TR* flow monitor. The reaction products were identified by HPLC comparison with unlabeled material using *Method A* (0 to 20% MeCN/0.1% CF_3COOH over 30 min). Reversed phase HPLC analyses: *Zorbax RX C18* analytical column (4.6 × 250), heated to 30° with a 10 min concluding wash with 100% MeCN. Preparative HPLC purifications: *Zorbax RX C18* preparative column (22.5 × 250). ^{1}H- and ^{13}C-NMR: *Varian U-400* spectrometer. LC/MS: *Agilent 1100 series LC/MSD* with an *Eclipse XCB C18* column (3.5 μm; 3.0 × 150 mm; solvent *A:* 20 nM of ammonium formate in H_2O; solvent *B:* MeCN; 5% *B* to 95% *B* in 10 min, then 5 min, at 100% *B*, followed by 5 min 5% *B*); in *m/z*.

General Procedure for the Synthesis of the [4.3.0]Pyrimidino Heterocycles **4**, **6**, **8**, **10**, *and* **12** via *Cyclization of the β-Aminocarboxamides.* A solvent-free solid mixture of β-aminocarboxamide (0.5 mmol) and dried sodium formate (34 mg, 0.5 mmol) was heated to 190° under N_2 for 2 h, and then cooled to r.t. After addition of a additional equiv. of sodium formate (34 mg, 0.5 mmol), the mixture was stirred with a spatula for 5 min, and heated to 190° under N_2 for 1 h. This procedure was repeated once more so that a total of 3 equiv. of sodium formate (1.5 mmol) were added. Overall heating time was 4 h. HPLC (*Method A*) and LC/MS assays showed that most of the starting material was consumed and the desired product was formed. H_2O (2 ml) was added, and the crystalline product was collected by filtration, followed by drying under high vacuum overnight. The yields for the isolated compounds are listed in the *Table.*

*Synthesis of 1,5-Dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one* (**4**) [7]. *5-Amino-1*H*-pyrazole-4-carboxamide hemisulfate* (**3**; 89 mg, 0.5 mmol) and sodium formate (35 mg × 3 = 105 mg, 1.5 mmol) gave **4** (53 mg, 89% based on **3**). IR (KBr): 1735, 1721, 1632, 1458. ^{1}H-NMR ($CDCl_3$): 5.1 (br. *s*, 1 H); 7.53 (*s*, 1 H); 8.13 (*s*, 1 H); 11.2 (br. *s*, 1 H). ^{13}C-NMR ($CDCl_3$): 98.8; 133.9; 148.3; 155.6; 157.9. ESI-MS: 137.2 ($[M+H]^+$).

*Synthesis of 1,9-Dihydro-6*H*-purin-6-one* (**6**). *5-Amino-1*H*-imidazole-4-carboxamide* (**5**; 63 mg, 0.5 mmol) and sodium formate (35 mg × 3 = 105 mg, 1.5 mmol) gave **6** (53 mg, 77% based on **3**). IR (KBr): 1735, 1613, 1450. ^{1}H-NMR ($CDCl_3$): 5.1 (br. *s*, 1 H); 7.53 (*s*, 1 H); 8.13 (*s*, 1 H); 11.2 (br. *s*, 1 H). ^{13}C-NMR ($CDCl_3$): 99.1; 132.9; 149.4; 155.0; 158.9. ESI-MS: 137.2 ($[M+H]^+$).

*Synthesis of 3-Methylisoxazolo[5,4-*d*]pyrimidin-4(5*H*)-one* (**8**). *5-Amino-3-methylisoxazole-4-carboxamide* (**7**; 71 mg, 0.5 mmol) and sodium formate (35 mg × 3 = 105 mg, 1.5 mmol) gave **8** (46 mg, 61% based on **7**). IR (KBr): 1731, 1668, 1410. ^{1}H-NMR ((D_6)DMSO): 2.42 (*s*, 3 H); 3.35 (br. *s*, 1 H); 8.21 (*s*, 1 H). ^{13}C-NMR ((D_6)DMSO): 22.4; 104.8; 149.0; 151.5; 160.1; 164.1. ESI-MS: 152.2 ($[M+H]^+$).

*Synthesis of 1,5-Dihydro-1-phenyl-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one* (**10**). *5-Amino-1-phenyl-1*H*-pyrazole-4-carboxamide* (**9**; 101 mg, 0.5 mmol) and sodium formate (35 mg × 3 = 105 mg, 1.5 mmol) gave **10** (86 mg, 81% based on the starting material **9**). IR (KBr): 1729, 1633, 1458. ^{1}H-NMR ((D_6)DMSO):

3.30 (br. *s*, 1 H); 7.36 (*t*, *J* = 7.6, 1 H); 7.53 (*m*, 2 H); 7.98 (*d*, *J* = 7.6, 2 H). ^{13}C-NMR ((D$_6$)DMSO): 108.3; 122.4; 127.8; 129.9; 136.7; 138.9; 149.5; 152.5; 157.9. ESI-MS: 213.2 ([*M* + H]$^+$).

*Synthesis of 5-Methyl-6-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one* (**12**). *2-Amino-4-methyl-5-phenylthiophene-3-carboxamide* (**11**, 116 mg, 0.5 mmol) and sodium formate (35 mg × 3 = 105 mg, 1.5 mmol) gave **12** (75 mg, 62% based on the starting material **11**). IR (KBr): 1730, 1613, 1449. ^{1}H-NMR ((D$_6$)DMSO): 2.46 (*s*, 3 H); 3.28 (br. *s*, 1 H); 7.38 (*m*, 2 H); 7.46 (*m*, 3 H); 8.05 (*s*, 1 H). ^{13}C-NMR ((D$_6$)DMSO): 15.1; 128.7; 129.6; 133.4; 146.3; 158.8; 158.9; 163.7; 163.7. ESI-MS: 243.2 ([*M* + H]$^+$). Anal. calc. for $C_{13}H_{10}N_2OS$: C 64.44, H 4.16, N 11.56; found: C 64.88, H 4.45, N 11.87.

*Synthesis of 1,5-Dihydro-4*H*-[6-^{14}C]pyrazolo[3,4-*d*]pyrimidin-4-one* (**4***). The reaction was carried out according to the *General Procedure* described above. Compound **3** (89 mg, 0.5 mmol) and sodium [^{14}C]formate (35 mg × 3 = 105 mg, 1.5 mmol, 80 mCi) gave **4*** (48 mg, 0.345 mmol, 18.3 mCi, *i.e.* 53 mCi/mmol). The chemical yield was 69% based on the limiting reagent **3**, whereas the radiochemical yield was 23% based on three equivalents of radiochemical reagent. Radiochemical purity for **4*** was 99% (*Method A*). ^{1}H-NMR ($CDCl_3$): 5.1 (br. *s*, 1 H); 7.53 (*s*, 1 H); 8.13 (*s*, 1 H); 11.2 (br. *s*, 1 H). ^{13}C-NMR ($CDCl_3$): 99.1; 132.9; 155.0; 158.9. ESI-MS: 138.3 ([*M* + H]$^+$).

*Synthesis of 4-Chloro-[6-^{14}C]1*H*-pyrazolo[4,3-*d*]pyrimidine* (**13**). $POCl_3$ (40 µl, 0.44 mmol) was added to a soln. of **4*** (18 mCi, 0.036 mmol) in CH_2Cl_2 (1 ml), and the resulting mixture was stirred at r.t. for 16 h. Pyridine (100 µl) was added, and the mixture was purified by flash chromatography (FC; SiO_2, CH_2Cl_2) to give **13** (46.3 mg, 15.84 mCi, 88%). ^{1}H-NMR (CD_3OD): 7.55 (*s*, 1 H); 8.40 (*s*, 1 H). ESI-MS: 157.2 ([*M* + H]$^+$).

Synthesis of N*-([6-^{14}C]1*H*-Pyrazolo[3,4-*d*]pyrimidin-4-yl)benzamide* (**16b**). A soln. of NH_4OH (6M in H_2O, 0.5 ml, 3 mmol), **13** (46.3 mg, 15.84 mCi, 0.3 mmol) and THF (1 ml) was heated at 85° for 2 h. After cooling to r.t., the aq. layer was extracted with CH_2Cl_2 (25 ml × 2). The combined org. layers were dried ($MgSO_4$), filtered, and concentrated under vacuum to give **16a** (= *[6-^{14}C]1*H*-pyrazolo[3,4-*d*]-pyrimidin-4-amine*, 11.25 mCi, 71%). ESI-MS: 138.2 ([*M* + H]$^+$). Benzoyl chloride (0.25 ml, 2.1 mmol) and pyridine (0.20 ml, 0.25 mmol) were added to a soln. of **16a** (11.25 mCi, 0.21 mmol) in CH_2Cl_2 (2 ml). The mixture was stirred at r.t. for 4 h and then purified by FC (SiO_2, 5% AcOEt in CH_2Cl_2) to give **16b** (48 mg, 10.6 mCi, 94%). ^{1}H-NMR (CD_3OD): 7.33–7.48 (*m*, 3 H); 7.60 (*s*, 1 H); 7.72–7.99 (*m*, 2 H); 8.30 (*s*, 1 H). ESI-MS: 242.3 ([*M* + H]$^+$).

Synthesis of N*-[1-[(1*R*,4*S*)-4-Hydroxycyclopent-2-en-1-yl]-[6-^{14}C]1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl]benzamide* (**17**). A suspension of **16b** (48 mg, 10.6 mCi, 0.2 mmol), $(Ph_3P)_4Pd$ (46.2 mg, 0.04 mmol), Ph_3P (26.2 mg, 0.1 mmol), DMSO (0.1 ml), and THF (1 ml) was stirred under N_2, while $NaN(TMS)_2$ (1.0M, 0.2 ml) was added. After being stirred at r.t. for 1 h, (1*R*,4*S*)-4-hydroxycyclopent-2-en-1-yl acetate [7] (40 mg, 0.24 mmol) was added. The mixture was heated to 60°, and stirred for 16 h. HCl (6M, 1 ml) was added, and the mixture was purified by FC (SiO_2, 15% AcOEt in CH_2Cl_2) to give compound **17** (29 mg, 4.8 mCi, 44%). ^{1}H-NMR (CD_3OD): 2.39–2.44 (*m*, 1 H); 2.52–2.63 (*m*, 1 H); 3.99–4.11 (*m*, 1 H); 4.39–4.51 (*m*, 1 H); 5.48–5.64 (*m*, 2 H); 7.38–7.45 (*m*, 4 H); 8.01–8.09 (*m*, 2 H); 8.39 (*s*, 1 H). ESI-MS: 324.2 ([*M* + H]$^+$).

Preparation of 8-Aza-7-deaza-[2-^{14}C]5′-noraristeromycin (= *(1*S*,2*R*,3*S*,4*R*)-4-(4-Amino-[6-^{14}C]1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)cyclopentane-1,2,3-triol*; **15**). *N*-Methylmorpholine *N*-oxide (NMO, 10.1 mg, 0.1 mmol) and polymer-bound OsO_4 (0.3 mmol/g, 5 mg, 0.0015 mmol) were added to a soln. of **17** (29 mg, 4.8 mCi, 0.09 mmol) in THF (1 ml) and H_2O (0.5 ml). The mixture was stirred at r.t. for 2 h. The solid was filtered off, and the soln. was treated with NH_4OH (6M, 1 ml) and MeOH (1 ml). The progress of the reaction was monitored by HPLC. After 5 h, TFA was used to adjust to pH 4. The mixture was concentrated, and then purified by prep. HPLC (*Zorbax RX C18*, 21.5 × 250; *A*, H_2O + 0.1% TFA; *B*, MeCN; *A*/*B* 77:23 (isocratic)) to give **15** (2.5 mCi, *i.e.* 53 mCi/mmol, 51% yield, 99.6% radiochemical purity). This result is in agreement with that reported by *Kitade* and co-workers [4a]. ^{1}H-NMR ((D$_6$)DMSO): 1.62–1.89 (*m*, 1 H); 2.22–2.62 (*m*, 1 H); 3.75 (br. *s*, 1 H); 3.90 (br. *s*, 1 H); 4.32–4.49 (*m*, 1 H); 4.75 (br. *s*, 1 H); 4.95 (*m*, 1 H); 5.05 (br. *s*, 1 H); 7.65 (*s*, 2 H); 8.12 (*s*, 2 H); 8.15 (*s*, 1 H). ^{13}C-NMR ((D$_6$)DMSO): 35.7; 59.6; 73.1; 74.5; 76.8; 100.1; 131.6; 153.1; 155.4; 157.8. ESI-MS: 254.2 ([*M* + H]$^+$).

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